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FURTHER STUDIES ON THE DEVELOPMENT OF THE SPORO CYST STAGES OF PLAGIORCHIID TREMATODES¹

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As a part of a program of investigations on the embryology of the digenetic trematodes, a study was made, during the summer of 1941 (Cort and Olivier, 1943b), at the University of Michigan Biological Station of the development of the sporocyst stages of a common plagiorchiid, *Plagiorchis muris* Tanabe, 1922. The material was obtained from immature natural infections in juveniles and adults of *Stagnicola emarginata angulata* (Sowerby) from a beach where a very high incidence of *P. muris* was known to occur.

Only late stages of the mother sporocyst of *P. muris* were found. They are entirely different from any mother sporocysts previously described for this group. They are oval, irregularly disc-shaped masses, 0.5 to 1.7 mm in largest diameter, which are made up of large numbers (about 300 to 500) of closely packed daughter sporocyst embryos (i.e., Figs. 1 and 2). These masses are discrete structures firmly attached to the outside of the snail's intestine, and may occur anywhere along its whole length. Each daughter sporocyst in the mass is surrounded by an outer coat of irregular cells. In fact, the mother sporocyst, at this stage, has no special outer wall, but is composed entirely of the daughters, which appear to be held together by the cells of their outer coats which form the matrix of the mass. This outer coat of the daughter sporocysts persists throughout their whole life. It has been described for mature daughter sporocysts of a number of species of plagiorchiiids, and has been called the paletot.

In the largest mother sporocysts the daughters are elongate and mobile when freed. Each contains cercarial embryos of different stages of development, and a single large discrete germ-mass (i.e., Fig. 13). It was found that when the mother sporocysts are located on those portions of the snail's intestine in front of the digestive gland, the daughters break away from the mother and migrate to the digestive gland and other organs of the snail, leaving behind no trace of the mother. When the daughter sporocysts cease migrating they become firmly attached to the snail's tissue, grow thicker and appear crowded with cercarial embryos. Soon, thickened areas of the true sporocyst wall appear at their ends, which frequently

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¹ A contribution from the University of Michigan Biological Station and the Department of Parasitology, School of Hygiene and Public Health, the Johns Hopkins University. This paper is one of a series on the embryology of digenetic trematodes in the intermediate hosts. The others have dealt with the strigeids (Cort and Olivier, 1941), the schistosomes (Cort and Olivier, 1943a; Cort, Ameel, and Olivier, 1944) and the plagiorchiiids (Cort and Olivier, 1943b).

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have invaginations at their centers giving the appearance of suckers. From these areas of growth, protrusions push out. Enlargement of these protrusions and their occupation by cercarial embryos produce a variety of irregularly shaped sporocysts, each enclosed in a paletot (l.c., Figs. 18-26). Later, when the period of growth is over, the daughter sporocysts become less irregular in shape because cercarial embryos have come to fill all their length. Growth in size continues after the escape of the first mature cercaria from the terminal birth pore. The germ-mass persists throughout the whole life of the sporocyst, and produces a constant stream of cercarial embryos by a process that has been interpreted as a special type of polyembryony, like that described for the strigeids (Cort and Olivier, 1941). Even in the oldest daughter sporocysts in old snails the germ-masses show no sign of exhaustion and cercarial embryos in all stages of development are present (l.c., Fig. 30).

These observations on the mother sporocyst and the development of the daughter sporocysts of *P. muris* do not fit in with any previous information in the literature on the development of the plagiurchiids. It was difficult to imagine the stages of metamorphosis of the miracidium that would produce the mass of daughter sporocyst embryos which we interpreted as a late stage of the mother sporocyst. No descriptions of sporocysts in this group were found resembling the migrating daughter sporocyst stages; and there were only a few scattered observations that suggested anything like the growth stages. Brooks (1930) stated that germ-masses were present in the daughter sporocysts of the plagiurchiids that he studied, but nothing in his description or figures resembles the germ-masses in these stages of *P. muris*. Certainly, further studies seemed to be badly needed to find earlier stages of the mother sporocysts of *P. muris* and to discover whether the development of other plagiurchiid species in the intermediate host is like this species. Therefore, we decided to continue during the summer of 1942 the study of natural infections of *P. muris* and to extend our observations to other plagiurchiid species.

The present paper, then, covers the observations of the summer of 1942 on the development of the sporocysts of the plagiurchiids. It seems wise to publish them at this time although the information on some of the species is rather fragmentary, since they confirm and supplement the results outlined above already published on *P. muris* (Cort and Olivier, 1943b). Also, it may be some time before it will be possible to undertake further investigations of this type. Altogether, six species of plagiurchiids were studied. By further examinations of *S. e. angulata* we obtained younger stages of the mother sporocysts of *P. muris* and some additional information on its daughter sporocysts. From this same species of snail some data were obtained on sporocyst development in *P. proximus* Barker, 1915, and *P. micracanthos* Macy, 1931. From a limited collection of adults of *Stagnicola palustris elodes* (Say) a few observations were made on the development of another plagiurchiid, *Cercaria talboti* McMullen, 1938. Finally, we examined a considerable series of *Helisoma trivolvis* (Say) and *Helisoma campanulatum smithii* (Baker) and studied the immature infections of *Macroderoides typicus* (Winfield, 1929) and *Alloglossidium corti* (Lamont, 1921) found in these hosts. From these studies, it is clear that in all six species the development of mother and daughter sporocysts has certain fundamental resemblances to that of *P. muris*; also, it is now possible to understand much more clearly the method of development of the mother sporocyst. Starting with the new

information on *P. muris* we will give the results on each species and then discuss the general picture which they give of the mother sporocysts and the development of daughter sporocysts in the superfamily Plagiorchioidea.

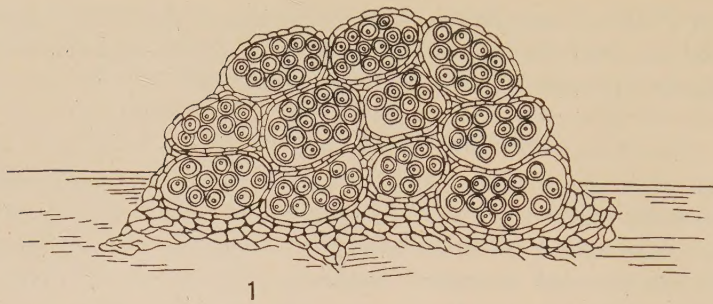
Plagiorchis muris Tanabe, 1922

An attempt was made in early August of 1942 to find very young mother sporocysts of *P. muris*, by examining a series of juveniles of *S. e. angulata* from the same beach on Burt Lake where the older mother sporocysts of this species had been found during the previous summer. Infections with *P. muris* were evidently entering these snails in large numbers since a large series of mother sporocysts was obtained, including several very early stages.

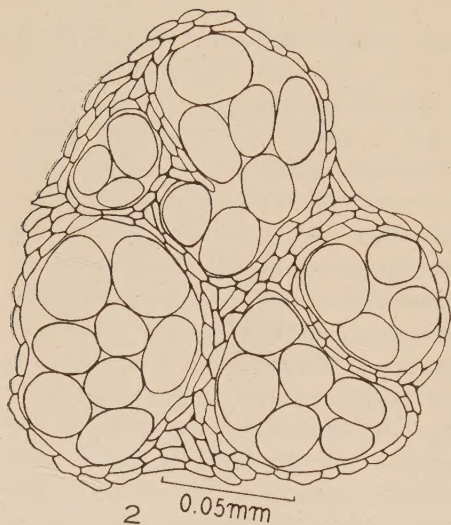
The youngest mother sporocyst of *P. muris* was a small mass about 0.48 by 0.38 mm in size adhering to the outer surface of the intestine of the snail. Careful manipulation and study under high power of the microscope gave an idea of its composition and made it possible to reconstruct a drawing of an optical section (Fig. 1). It was divided into lobes each containing a number of germ cells. The cells forming the walls were of the same type as those forming the paletots of the individual daughter sporocyst embryos in the older mother sporocysts. Several layers of these same cells formed a base for the attachment of the sporocyst to the outside of the intestine. Some of the compartments containing the germ cells were completely enclosed by the wall while others were only partly enclosed. One other mother sporocyst was found in which only germ cells were present. It was considerably larger, 1.05 by 0.60 mm, and had the same structure as the one figured.

A slightly older mother sporocyst appeared to be divided into several lobes each of which was subdivided into smaller compartments, containing both single germ cells and embryos in the cleavage stages. In some places ingrowths from the wall could be seen which appeared to be the beginnings of still further subdivisions. That is, the whole mother was divided into primary divisions which appeared as lobes, each lobe was subdivided into secondary compartments, and the beginning of tertiary divisions could be seen. In another mother sporocyst in which the subdivision had reached about the same stage, no single germ cells were present but only embryos in the cleavage stages (4- to 16-cell).

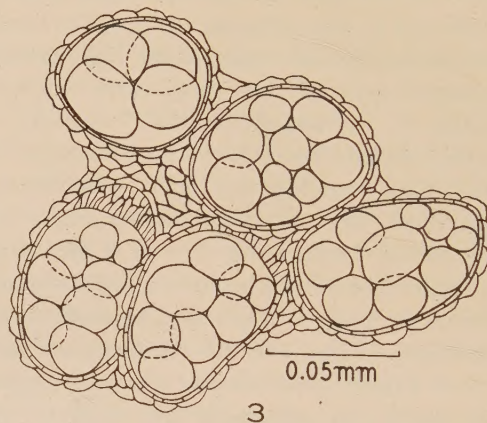
In another slightly older mother all the germinal material was in the round "germ ball" stage and the compartments each contained from two or three to several of these embryos. Fig. 2 shows a small part of this mother sporocyst. It shows definite evidence of the growing in of the wall to subdivide the compartments. In some places the wall is more than one layer of cells in thickness. In slightly more advanced stages some compartments contained several free embryos, but most of the daughter sporocyst embryos were surrounded by paletots formed from the wall. In all later stages, the subdividing of the mother by the ingrowing of the wall had been completed and each daughter sporocyst embryo was enclosed in a paletot. Thus the outer layer of cells of the daughter sporocysts, the paletot, which persists throughout their whole lives is clearly seen to be derived from the mother sporocyst wall. Fig. 3 shows a group of daughter sporocyst embryos each surrounded by a paletot. The mother sporocyst from which this drawing was made was about the same stage as the youngest seen during the summer of 1941 (Cort and Olivier, 1943b, Fig. 1). In further development, as described in the earlier paper, the daughter sporocysts



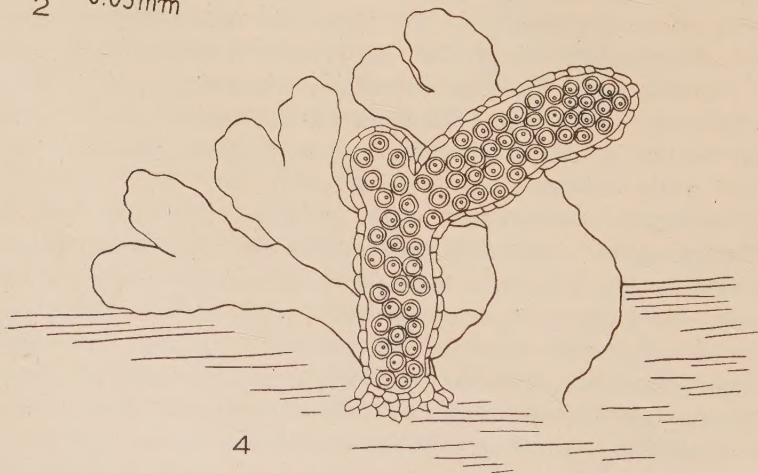
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FIG. 1. Very young mother sporocyst of *Plagiorchis muris*.

FIG. 2. A portion of a somewhat older immature mother sporocyst of *Plagiorchis muris*.

FIG. 3. A small portion of an older immature mother sporocyst of *Plagiorchis muris* showing daughter sporocyst embryos already enclosed in their paletots.

FIG. 4. Very immature mother sporocyst of *Plagiorchis proximus*.

elongate and increase in size, until, when they are ready to migrate, their paletots are quite separate, with only a few cells left between them. These observations then confirm those of the previous summer on the mother sporocyst of *P. muris* and show its method of development.

In a number of infections of *P. muris* studied during the summer of 1942, the mother sporocysts were on the parts of the intestine not in contact with the digestive gland. Infections were also found that confirmed the findings of the previous summer on the migrations of the daughter sporocysts from mothers, so located, to the digestive gland. Also, there were numerous cases in which the daughter sporocysts were in the peculiar growth stages. None of the observations in the second summer's work modified the conclusion that asexual reproduction of the daughter sporocysts by fission or budding does not normally occur.

In the summer of 1942, however, we found a number of infections in which the daughter sporocysts were in the growth stages without having migrated from the mother. In such cases it is evident that all chance of migration has been lost. Several of these infections will be described since they show interesting relations between growth and migration in the daughter sporocysts.

In one case a mother sporocyst was attached to the first part of the intestine not far from the stomach. Along its edge daughter sporocysts could be seen that were in process of breaking away; others had already started migration and a few had reached the digestive gland. However, only a part of the daughters was migrating and a large proportion of those still in the mother, perhaps approximately one half, had started to grow without migration. This snail contained no other larval trematode infection.

Another snail had a mature infection of *P. muris* which had the usual distribution all through the digestive gland. In addition there was a mother sporocyst attached to the intestine in front of the gland. The daughter sporocysts of this mother were less numerous than usual and all were in the growth stages. Thus with the liver fully occupied it appeared that the daughter sporocysts of the superimposed infection were developing without migration.

In another snail in which there was a mature infection of *Diplostomum flexicaudum*, a mass was found attached to the intestine in front of the digestive gland. It was composed of mature daughter sporocysts of *P. muris* containing fully developed cercariae. This was interpreted as a case in which the daughter sporocysts had completed their development without migrating from the mother.

In another infection a mass of mature, heavily pigmented daughter sporocysts was found attached to the first part of the intestine just outside the digestive gland. Not a single daughter sporocyst was found in the digestive gland or elsewhere in the tissues of this snail, and there was no other larval trematode infection. This mass had the general shape of a mother sporocyst, but was much larger. It was also interpreted as a case of the completion of the development of the daughters without leaving the mother.

In several infections the mother sporocysts were attached to the part of the intestine in contact with the digestive gland, with all the daughters in growth stages without any having migrated. Also, in one infection the mature daughter sporocysts were limited to a mass on the surface of the digestive gland. In this case the daughters had evidently completed their development in position without any

leaving the mother. In another case such a mass, equal in size to about 1/5 that of the digestive gland, was found on the gland with the cercariae almost mature. In some of the cases in which the daughter sporocysts developed without migration there were other larval trematode infections present and in others there were no other infections. Usually when the mother sporocysts were located on the surface of the digestive gland part of the daughters had migrated away from the mother and part had developed without migrations. In such infections the original position of the mother could be determined by finding a solid mass of daughters attached to each other. It is evident, therefore, that migration of the daughter sporocysts of *P. muris* from the mother is not necessary for further growth to take place. Development without migration seems to be rather rare when the mother sporocysts are located in front of the digestive gland, but appears to be the rule for part or even all of the daughter sporocysts when the mothers develop in contact with this gland.

Plagiorchis micracanthos Macy, 1931

P. micracanthos, the life history of which was worked out by McMullen (1937), is rather rare in *S. e. angulata* in the Douglas Lake region. No immature infections that could be identified as this species were found in our examinations. However, from the few mature infections that were observed, some evidence was obtained that the development of the mother and daughter sporocysts of this species is like that of *P. muris*. In the first place, the daughter sporocysts are very similar to those of *P. muris*, have well developed paletots, and have a similar distribution in the digestive gland and other tissues of the snail host.

One infection of this species was particularly interesting. It consisted of a large, solid mass of mature daughter sporocysts between the foot and digestive gland of a snail. This snail also had a mature infection of *P. muris* with the daughter sporocysts scattered throughout the gland. The mass of mature sporocysts of *P. micracanthos* was a solid discrete structure. It was roughly lens-shaped and the sporocysts which made up its entire structure were so tightly held together that it was removed without breaking. The paletots of these sporocysts were heavily pigmented, giving the whole mass an orange color. These sporocysts contained numerous, active, fully developed cercariae. The interpretation of this mass as a mother sporocyst in which the daughters had developed to maturity without migration seems reasonable, since it resembled very strikingly the cases found in *P. muris* infections in which the daughter sporocysts had completed development without leaving the mother. This certainly suggests that the mother sporocyst of *P. micracanthos* is like that of *P. muris*. Also, the distribution of the daughter sporocysts in other infections of this species suggests that they usually escape from the mother and migrate like those of *P. muris*.

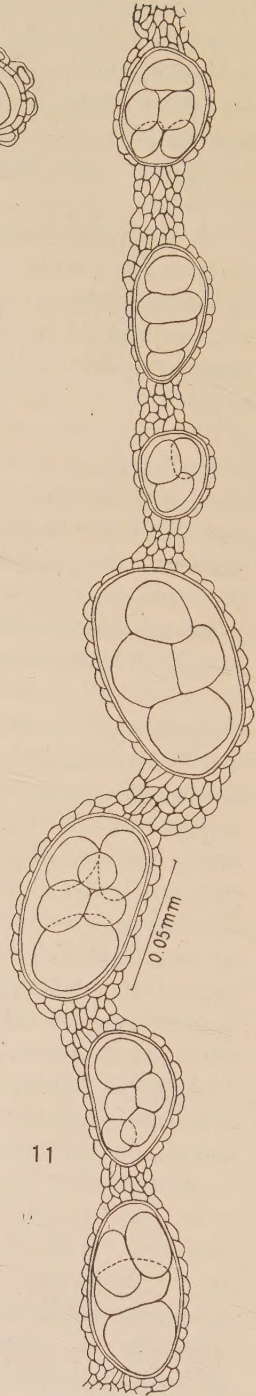
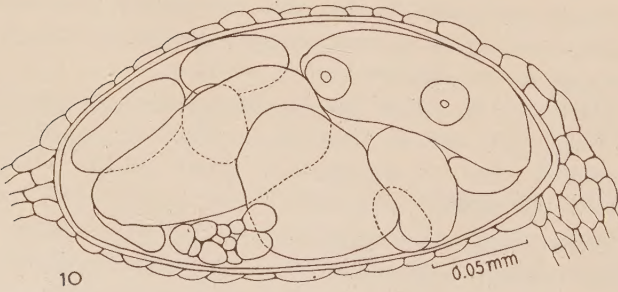
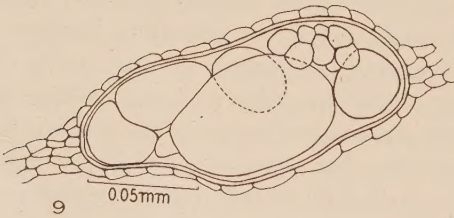
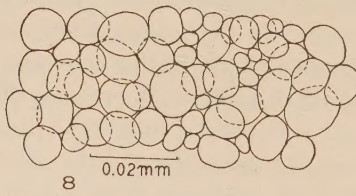
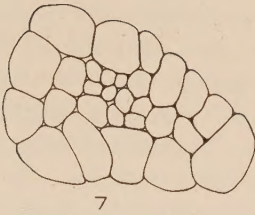
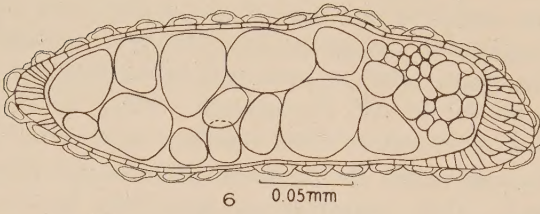
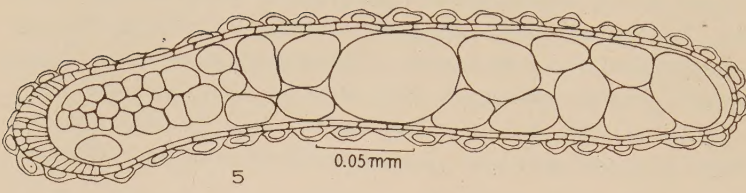
Cercaria talboti McMullen, 1938

From a collection of *S. p. elodes* from Black Lake, Michigan, we were able to make some observations on the sporocysts of *Cercaria talboti*, which was described by McMullen (1938) from this host and *S. e. angulata*. In several mature and old infections the daughter sporocysts appeared somewhat larger than the measurements given by McMullen (0.795 by 0.188 mm); in one of the oldest infections three of the largest sporocysts had lengths of 1.4, 2.1 and 2.4 mm. The distribution of the

mature daughter sporocysts of this species in the snail host is like that of *P. muris*. They have well-developed paletots containing orange pigment, which is very dense in the oldest infections. Large germ-masses are present, composed of large numbers of both unicellular and multicellular components. These germ-masses are larger than those in the mature daughter sporocysts of *P. muris* and contain more components (Fig. 8). Two that were measured had sizes of 0.17 by 0.07 mm and 0.16 by 0.10 mm. More than one germ-mass was never found in a single daughter sporocyst.

In the snails from this collection two immature plagiorchiid infections were found. They were considered to belong to *C. talboti* since this was the only xiphidiocercaria in this collection and their germ-masses were like those of the mature daughter sporocysts of that species. In one of these infections the daughter sporocysts were in the migrating stage. Part of them was in an irregular mass attached to the snail's intestine not far beyond the stomach. Those at the edge of this mass appeared to be about to migrate. Other daughter sporocysts in the migrating stage were present on and among the organs in front of the digestive gland and all through this gland. Their migration had apparently been recent since none was in the growth stages, and only a few had become attached. The number of daughter sporocysts appeared to be greater than in infections of *P. muris*. A partial count of those still in the mass on the intestine gave 326; and it was estimated that the total number both in the mass and scattered in the tissues may have been a thousand or even more. As seen in Fig. 5, the migrating daughter sporocysts of *C. talboti* are very similar to those of *P. muris*. The cells of the paletot were very distinct, making the surface of the sporocysts quite irregular. Each migrating daughter sporocyst contained from 11 to 18 cercarial embryos and a single germ-mass located at one of the ends. The largest of the cercarial embryos was located at about the middle of the sporocyst, and showed no visible differentiation of organs. These sporocysts were larger than the migrating daughter sporocysts of *P. muris*; eleven that were measured had lengths of from 0.4 to 0.9 mm. Their germ-masses were also distinctly larger than those of the migrating daughter sporocysts of *P. muris*. They had the same structure as in the mature daughter sporocysts but were somewhat smaller; five that were measured had lengths ranging from 0.11 to 0.14 mm, and widths from 0.09 to 0.11 mm. In comparing this immature infection of *C. talboti* with those of *P. muris* at the same stage it seems evident that the method of development must be the same and that the mass from which the daughter sporocysts were migrating must represent the final stages of a mother sporocyst of the same type as that described for *P. muris*.

In the other immature infection of *C. talboti* the daughter sporocysts appeared to be just beyond the migrating stage, and no trace of a mother sporocyst mass remained. They were distributed widely in the digestive gland and also on and among the organs in front of that gland. The cells of the paletot were very prominent and there were distinct thickened areas of the true sporocyst wall at the ends (Fig. 6). These immature daughter sporocysts were attached to the tissues of the host, and were thicker and shorter than the migrating stages of the other infection, the width being about one-third their length. There was also an increase in some of them in the bulk of the germinal material, which appeared more crowded than in the migrating stage (Fig. 5). In such sporocysts the germ-masses were



very prominent, sometimes having a length equal to about one-third that of the body cavity. No growth stages were present in this infection, but the similarity to the pregrowth stages of the daughter sporocysts of *P. muris*, and especially the thickenings of the sporocyst wall at the ends, suggested the same type of growth in the two species. In fact the similarity to *P. muris* was so great that it seems almost certain that the development of the mother and daughter sporocysts of *C. talboti* is like that species.

Alloglossidium corti Lamont, 1921

The life cycle of *A. corti* has been studied by McCoy (1928), McMullen (1935) and Crawford (1937). These reports, however, give very little information on the sporocysts. Crawford described the daughter sporocysts as a "mass of much elongated cylindrical sacs twisted around each other." He gave the measurements of a typical sporocyst as 1.8 by 0.16 mm.

In our studies of the development of the sporocysts of this species from *H. trivolvis* and *H. c. smithii* we first examined a number of mature infections. The daughter sporocysts varied considerably in size and shape but were usually more elongate than those of *P. muris*, *P. micracanthos*, and *C. talboti*. In one mature infection seven sporocysts taken at random measured from 0.79 to 2.03 mm in length and from 0.23 to 0.30 mm in width; in another infection twenty were measured, giving lengths of from 0.50 to 2.33 mm and widths from 0.20 to 0.29 mm; the largest sporocyst that was measured from any infection was 3.72 by 0.30 mm. It can be seen from these measurements that the variation in width is small, but that in length is surprisingly great. This shows that average length measurements for the daughter sporocysts of this species would have no significance as a specific character, and suggests that growth occurs chiefly from the ends. As would be expected, the number of active cercariae and developing embryos present in a sporocyst varies with its size; in one small sporocyst, 0.79 by 0.24 mm, 8 active cercariae and four developing embryos were counted, and in one 2.03 by 0.30 mm there were 21 active cercariae.

The germ-masses of the mature daughter sporocysts of *A. corti* are fairly large, complicated structures composed of considerable numbers of both unicellular and multicellular components (Fig. 7). Although they varied considerably in shape, they are roughly triangular, and quite flat. It was difficult to measure or draw them because they were pushed around so much by the active movements of the cercariae. The largest measured had a length of 0.2 mm; another had a size of 0.16 by 0.10 mm and two smaller ones had sizes of 0.13 by 0.05 mm and 0.13 by 0.07

FIG. 5. Daughter sporocyst of *Cercaria talboti* in the migrating stage.

FIG. 6. Immature daughter sporocyst of *Cercaria talboti* just after attachment in the digestive gland of the snail intermediate host.

FIG. 7. The germ mass from a mature daughter sporocyst of *Alloglossidium corti*.

FIG. 8. Camera lucida drawing of an optical section of a germ mass from a mature daughter sporocyst of *Cercaria talboti* to give an idea of the large numbers of components included.

FIG. 9. Immature daughter sporocyst of *Macroderoides typicus* showing the relations of the paletot, the small number of cercarial embryos, and the characteristic germ mass.

FIG. 10. Immature daughter sporocyst of *Macroderoides typicus* at a later stage than Figure 9.

FIG. 11. Showing a portion of a tubule of the mother sporocyst of *Macroderoides typicus* containing immature daughter sporocyst embryos. Over 40 embryos were counted in this one tubule.

mm, respectively. Usually as shown in the drawing (Fig. 7) the multicellular components are around the periphery and the unicellular components are massed in the center. No more than one germ-mass was ever seen in a sporocyst and it was not always easy to find them in the individual sporocysts because of the large numbers of active cercariae and developing embryos that were present.

A well-developed paletot containing orange pigment which is particularly dense in the oldest infections is present around the mature daughter sporocysts of *A. corti*. In most of the sporocysts the end where the birth pore is located is definitely more attenuated than the other. The pore could be clearly seen only when cercariae were escaping. The number of daughter sporocysts in individual infections is very large. In one case 3186 were actually counted, which probably fell far short of the true number.

Although the daughter sporocysts in mature infections of *A. corti* are sometimes found on and among the organs in front of the digestive gland, they appear to be much more localized in this gland than in infections with *P. muris*. Sometimes they are scattered widely through the digestive gland, but frequently they are found in solid masses imbedded in the gland or on its surface. Sometimes, all the sporocysts in the infection would be concentrated in one mass. When the daughter sporocysts are in such masses, they are tightly attached to each other by their paletots and are entirely separate from the tissue of the digestive gland. No masses of mature sporocysts were ever found in front of the digestive glands as was reported above for *P. muris* and *P. micracanthos*. These masses of mature sporocysts of *A. corti* are similar to those sometimes found in or on the digestive gland in *P. muris* infections, which were interpreted as daughters which had developed to maturity without leaving the mother.

The general localization of the mature daughter sporocysts of *A. corti* in the digestive gland of the snail host, and the finding of them in masses in or on the surface of this gland, suggested that the mother sporocysts of this species probably develop on that part of the snail's intestine in contact with the digestive gland. We, therefore, concentrated our search on this section of the intestine and found a considerable series of mother sporocysts. We considered that all of these mother sporocysts belonged to *A. corti*, because in the only other species of plagiurchiid, *Macroderoides typicus*, that we found in any of the collections of *Helisoma* studied, the mother sporocysts are very different in structure and are always in front of the digestive gland (*vide infra*).

The youngest mother sporocyst of *A. corti*, was a regular rounded mass about 2 mm in diameter, which was attached to the intestine. It was imbedded in the surface of the digestive gland but was pulled away unbroken with the removal of the intestine. When this mother sporocyst was carefully teased apart it was found to contain a large number of very immature daughter sporocyst embryos. These embryos did not appear to be in a series of compartments as in the very young mother sporocysts of *P. muris* but were in a general matrix of mother sporocyst cells. Some of them were in the 6 to 12 cell stages, and the largest were elongate, well-developed sporocysts approaching the migrating stage. The smallest was about 0.027 mm in diameter while the largest was 0.110 by 0.060 mm. All gradations between these two sizes were present. In fact, we found some separate germ cells in a matrix of mother sporocyst cells, but could not be sure that they had not come from small

embryos that had broken up. Very immature embryos in the round "germ ball" stage, only 0.050 to 0.070 mm in diameter, had clearly defined paletots.

Two somewhat larger mother sporocysts of *A. corti* were found in two other snails attached to the intestine after it was pulled away from the digestive gland. They contained daughter sporocyst embryos varying from round "germ balls," 0.06 to 0.07 mm in diameter, to elongate forms up to 0.32 mm in length containing well developed cercarial embryos. All the embryos examined from these two mothers had definite paletots. In the youngest the paletot consisted of only a few of the mother sporocyst cells scattered over the surface, but in some still in the round "germ ball" stage it was a distinct layer. As in the younger mother sporocyst described above, no organization into compartments or tubes could be made out that suggested the method of development.

In a somewhat older infection a part of the mother sporocyst mass was still attached to the intestine after it was pulled away from the digestive gland. Daughter sporocyst embryos in this mass varied from round "germ balls" to elongate forms that were active and ready to migrate. All had definite paletots and germ-masses could be seen in the largest elongate forms, which resembled closely the same stages described for *P. muris*. Some migrating daughter sporocysts were found in the digestive gland at a distance from the mother. Other immature daughters in the gland had become fixed and had started to grow. The growth stages are like those of *P. muris* (Cort and Olivier, 1943b). They include thickened forms with sucker-like growth areas at the ends, forms with small projections from the centers of the growth areas, and others divided into two to three parts showing bizarre shapes. In fact, the shapes depicted for the growth stages of *P. muris* (l.c., Fig. 31) with a variety of modifications could have been drawn from this one infection.

In several other infections of *A. corti* daughter sporocysts in the growth stages were also found. In some cases, it was evident that all the sporocysts had migrated away from the mother before starting to grow. In others, daughters in growth stages were scattered through the digestive gland, and some were in a mass that appeared to be a remnant of the mother. In still others, only the mother mass was present with some or all the daughter sporocysts in the growth stages. For example, in one infection the whole tip of the digestive gland appeared to be made up of a solid mass of immature daughter sporocysts attached to the intestine, most of which were already in the growth stages. Only a very few sporocysts had migrated away from this mass. In another infection the sporocysts were all in one large mass attached to the intestine. Most of them seemed to be in the growth stages, and the cercariae in some of them had their suckers and tails well differentiated.

The evidence from both mature and immature infections of *A. corti* indicates, therefore, that the mother sporocysts of this species develop attached to that part of the snail's intestine that is in contact with the digestive gland. Thus, as they grow in size they tend to become imbedded in the digestive gland. When the daughter sporocysts reach the migrating stage they may leave the mother and establish themselves elsewhere in the gland, and a few may even migrate to tissues in front of the gland. There is, however, a definite tendency for the daughter sporocysts of this species to develop without leaving the mother. This would account for the finding of masses of daughter sporocysts in various stages of growth rather deeply imbedded in the digestive gland but separate from its tissue.

Unfortunately, we did not find early enough stages of the mother sporocysts of *A. corti* to show just how development proceeds. It is evident, however, that this early development must differ from that of *P. muris* since in *A. corti* the daughters in the same mother vary greatly in the stage of development. Also, no evidence was found of the formation of compartments by the growing in of walls. In *A. corti* it appears that the paletots of the daughter sporocysts are formed by the attachment to them of cells of the matrix of the mother sporocyst in which they lie.

Plagiorchis proximus Barker, 1915

The larval stages of *P. proximus* are very common and widespread in the Douglas Lake region in *S. e. angulata*. The life cycle of this species was worked out by McMullen (1937) who gave an average size for the daughter sporocysts of 1.021 by 0.243 mm, and noted that they resemble those of *P. muris*. We did not make studies of mature daughter sporocysts of *P. proximus*, except to note that they had well developed pigmented paletots and contained germ-masses. Also, in one case it was noted that sporocysts of this species containing mature cercariae were arranged in strings with the individual sporocysts connected with each other by extensions of the paletots.

In the examinations of *S. e. angulata* during the summers of 1941 and 1942, which were made in connection with the studies on *P. muris*, we found a number of immature plagiorchiid infections in which the daughter sporocysts were arranged in strings which showed branching. Mother sporocysts were also found which were composed of branched tubules containing embryos. All these were considered to belong to *P. proximus* since it was the only plagiorchiid species, besides *P. muris*, that has ever been found in any numbers in this host from the area where the collections had been made. Also, as noted above, mature sporocysts of *P. promixus* had been found in strings. In addition, *P. micracanthus* and *C. talboti*, which have been collected from this host in this area a few times, appear to resemble *P. muris* in the method of mother sporocyst development.

The youngest mother sporocyst which we assigned to *P. proximus* was attached to the snail's intestine in front of the digestive gland. It consisted of four irregular tubules, attached at a common base, each of which had begun to branch dichotomously (Fig. 4). The tubules were about 0.1 mm in diameter, and the whole mass was over 0.5 mm in diameter, which was about the same as the length of each tubule. The walls were composed of a single layer of cells and the same cells formed the base for attachment. These cells are like those that form the wall of the youngest mother sporocyst of *P. muris* described above (Fig. 1). The tubules of this mother sporocyst were packed with large numbers of germ cells which were exactly like those found in the youngest mother sporocysts of *P. muris*. In two places at the ends of the tubules there were small embryos in the early cleavage stages. This was the only very immature mother sporocyst of *P. proximus* that we found. It gives a clue to the method of development of the later stages that were present in considerable numbers.

Several somewhat later stages of the mother sporocysts of *P. proximus* were found attached to the intestine of the snails outside the digestive gland. In them the germinal material was in very early stages of embryonic development and numerous germ cells were still present. These mother sporocysts appeared to be of two types.

A few of them were bushy masses of branching tubules, attached at the base to the intestine. Most, however, were flattened plates extending from the intestine along the mesenteries. These plates appeared to consist of a single layer of branching tubules held together by the cells of their walls, which were later to form the paletots of the daughter sporocysts.

In the youngest of the bush-like mother sporocysts most of the germinal material consisted of germ cells which were packed into the narrow branching tubules in enormous numbers. In a number of the tubules there were early embryos up to about the 16-cell stage. The number of germ cells and embryos appeared to have very greatly increased as compared with the number of germ cells present in the youngest mother sporocyst described above. There seemed to be a great variation in the amount of germinal material present in different immature mother sporocysts, since in one case the germ cells and early embryos were comparatively few and scattered in a linear arrangement along the tubules. Another slightly older bush-like mother sporocyst also had all the germ cells and embryos arranged in linear fashion along the tubules. In it some of the embryos had a distinct sporocyst wall and contained cercarial embryos. When the tubules contained such young daughter sporocysts their walls had become the paletots of the embryos, which appeared as bulges connected by narrower regions. In some of the tubules of this mother sporocyst the germinal material consisted of embryos in cleavage stages arranged in linear fashion and in many of them it consisted only of scattered germ cells.

Most of the mother sporocysts of *P. proximus* which were found attached to the intestines of the snails outside the digestive gland were in plates, one tubule in thickness. In fact, none of the bush-like type contained daughter sporocysts in the later stages of development. It seems possible, therefore, that the mother sporocysts of this species as they develop tend to grow along the mesenteries and assume the flattened plate-like character. In what appeared to be the youngest of the plate-like type there were embryos in the germ ball stage, each surrounded closely by its paletot formed from the tubule wall. At this stage the tubules looked almost like strings of beads. In this mother, numbers of tubules still contained embryos in early cleavage stages, and in some the germinal material consisted only of separate germ cells. Older plate-like mother sporocysts are frequently quite extensive. One showed two distinct parts and extended quite a distance along the intestinal wall and out along the mesentery. Along its edges were daughter sporocysts, with a well developed wall, which contained cercarial embryos. Elsewhere the tubules were much narrower and contained only cleavage stages or separate germ cells.

Other older plate-like mother sporocysts had daughters, especially along the edges, in the migrating stage. One such mother, on the last part of the intestine outside the digestive gland, was irregularly oval in outline and migrating daughter sporocysts could be seen breaking away from its margin. It also contained younger embryos in all stages of development and in some of the smaller tubules there were only germ cells. Two mother sporocysts, attached to the intestine outside the digestive gland, even contained daughters in growth stages like those described for *P. muris* (Cort and Olivier, 1943b).

A few mother sporocysts of *P. proximus* were found attached to the portion of the intestine imbedded in the digestive gland. These appeared as masses of branching tubules with embryos in various stages of development. In one case such a mass

at the base of the digestive gland contained large numbers of daughter sporocysts already in growth stages. Although more observations are needed, it seems clear that in *P. proximus* daughter sporocysts may either migrate from the mother, or the growth stages may take place without migration. Very striking also in the mother sporocysts of *P. proximus* is the great variation in the stages of development of the embryos, since migrating daughters, and in a few cases even growth stages, and all earlier stages of embryonic development are found in sporocysts in which some tubules still contain germ cells.

Macroderoides typicus Winfield, 1929

In his discussion on the life cycle of *M. typicus*, McMullen (1935) gave no information on the sporocyst stages except that the mature daughter sporocysts have a size of about 0.36 to 0.13 mm and contain 1 to 4 cercariae. Our studies on the development of the sporocysts of this species were made of natural infections in *H. trivolvis* and *H. c. smithii*.

In mature infections the daughter sporocysts are in large orange-colored masses lying among the organs between the foot and digestive gland of the snails. In occasional infections the sporocyst mass would be in contact with the base of this gland, would somewhat overlap its proximal part or would even give the appearance of being partly imbedded in it. Never, however, are the sporocysts actually in the tissues of the digestive gland.

The daughter sporocysts of this species are comparatively small and in the mature infections the orange pigment in the paletot is unusually dense. When the masses of mature sporocysts were detached from the snail's organs and teased apart with needles they were usually found to be in strings, attached end to end by extensions of their paletots. Sometimes in the densest masses the arrangement into strings is not evident. The sporocysts themselves are by far the smallest that were studied and are irregularly oval with a slightly pointed end at which the birth pore is located. Measurements of twenty from one infection gave lengths ranging from 0.48 to 0.87 mm and widths from 0.20 to 0.36 mm. In other infections some were considerably smaller and some were larger. The two largest sporocysts that were seen in any of the numerous infections observed measured 1.34 by 0.23 mm and 0.95 by 0.23 mm, respectively. The sizes of the masses and the number of daughter sporocysts in them vary greatly in different infections. In one of the larger infections in which an attempt was made to count the sporocysts the number was found to be approximately 4000.

The number of mature and developing cercariae in the daughter sporocysts of *M. typicus* is much less than for any other of the plagiiorchiid species in our series, varying from 5 to 12 in eleven sporocysts from one infection; of these from 1 to 4 were mature or almost mature cercariae. In addition, each daughter sporocyst contains a small, somewhat flattened germ-mass of rather regular outline. It was not always easy to find these germ-masses because the contents of the sporocysts were moved about by the activity of the cercariae and they had to be carefully observed to distinguish them from cercarial embryos. The germ-masses in this species are the smallest of any of the plagiiorchiid species studied and the number of their components is much less. Seven that were measured within the living sporocysts had a range in length of from 0.070 to 0.105 mm and a width from 0.049 to 0.059 mm.

Even in the oldest infections, as already noted, some sporocysts are attached end to end in strings by extensions of their paletots. Daughter sporocyst embryos in the round "germ ball" stage are occasionally present in infections containing mature cercariae. Also, the same string may contain a few small, immature daughter sporocysts and others very much larger with mature or almost mature cercariae. Occasionally very long strings of a large number of sporocysts could be isolated. Even the very longest strings were unbranched. Figs. 9 and 10 show two stages of immature daughter sporocysts from such strings.

The finding of these strings containing immature daughter sporocysts led us to look carefully for young infections in which none of the cercariae was mature. Three immature infections were found at about the same stage of development in which the youngest embryos were small round "germ balls" and the largest although well formed sporocysts contained no cercariae with differentiated body and tail. These infections, which consisted of a mass of tubules or strings attached to the enlargement of the esophagus of the snails next to the stomach, were interpreted as mother sporocysts. From the central mass of the mother sporocyst strings extended out to considerable lengths reaching the nearby organs of the snail. The examination of these strings showed that they consisted of tubules of paletot cells enclosing at intervals the developing daughter sporocyst embryos. Some of them were surprisingly long; in one 40 daughter sporocyst embryos at various stages of development were counted (Fig. 11). We had the impression that some of the strings were even much longer than this. In no instance was any branching of these tubules seen.

A still younger mother sporocyst was found in another snail, also located on the bulb formed at the point where the esophagus enters the stomach. It was composed of three small masses of the tubules or strings radiating from a common center. All that could be made out when this whole mass was detached and observed under the microscope were large numbers of unbranched tubules of paletot cells each containing a linear series of the daughter sporocyst embryos. The oldest daughter sporocyst embryos in this mother had well-formed walls and the youngest were in early cleavage stages. Although careful search was made no germ cells were seen. At the tips of some of the strings, embryos in the cleavage stages or in the early "germ ball" stage were present, and the paletot cells could be seen to form definite tubules along which the embryos were scattered. In those parts of the strings where the embryos had grown to immature sporocysts, in which the wall was well formed and the cercarial embryos were distinct, the walls of the tubule adhered closely to the sporocysts forming their paletots.

DISCUSSION

The observations recorded in this and the previous paper (Cort and Olivier, 1943b) on the development of the plagiurchiids gives an entirely new conception of the mother and daughter sporocyst development in this group. In all of the six species studied the daughter sporocysts retain throughout their whole lives an outer layer of cells, the paletot, which is derived from the mother sporocyst. Characteristic also is the formation of large numbers of daughter sporocysts by multiplication of the cells of the germinal line in the early stages of the development of the mother sporocysts. Then, in the early development of the secondary germinal sacs, the

daughter sporocysts, there is first a limited multiplication of germ cells followed by the formation of a single germ-mass for each daughter. These germ-masses produce a constant stream of cercarial embryos throughout the whole life of the daughter sporocysts by the breaking off of multicellular components in a special type of polyembryony. The combination of the extensive multiplication of the cells of the germinal line in the mother sporocysts which is interpreted as a primary polyembryony of the zygote (fertilized ovum) with the secondary polyembryony of the germ-masses of the daughters, produces the enormous numbers of cercariae so characteristic of the plagiiorchiids. In a species like *A. corti* where the number of daughter sporocysts is unusually great, and each daughter is large, it seems possible that more than a million cercariae can be produced from one fertilized ovum.

Another striking point in the development of these plagiiorchiids is the almost unlimited growth of the cells forming the wall of the mother sporocyst which later form the paletots of all the daughter sporocysts. These cells appear to come from the "subepithelial" layer of cells of the miracidium which forms the wall of the young mother sporocyst. Although the wall of the mother sporocyst increases greatly in extent in other groups to form the comparatively huge sacs of the strigeids or schistosomes, its almost unlimited growth with the formation of the paletots of the daughter sporocysts is characteristic of the plagiiorchiids.

Another characteristic of this group is the peculiar method of growth employed by the daughter sporocysts after they have reached their final position in the snail's tissue. While this was seen in only three of the six species studied it probably occurs in all but *M. typicus*. This growth, consisting of the extension of the walls of the sporocysts from growth areas at their ends, produces stages with very bizarre shapes, which sometimes suggest multiplication by budding or fission. However, there is no evidence from our observations that this kind of asexual multiplication of daughter sporocysts normally occurs.

The discussion given above indicates the general pattern of plagiiorchiid development as shown by the six species studied. The most striking differences among them are in the structure of the mother sporocysts and in the methods by which the daughters are distributed to the tissues of the snail. In these respects the six species appear to show four different types.

The mother sporocyst of *P. muris* illustrates the first type, to which those of *P. micracanthos* and *C. talboti* also appear to belong. The youngest mother sporocyst of *P. muris* observed (Fig. 1) already was divided into compartments by the ingrowth of the cells of the wall. Each compartment contained a number of separate germ cells. By later ingrowths of the wall the mother sporocyst of this type is subdivided into smaller and smaller compartments, until finally each embryo is surrounded by a part of the wall, the paletot. The oldest mother sporocysts consist, therefore, of a mass composed of large numbers of elongate motile daughter sporocysts each surrounded by its part of the mother sporocyst wall. Then, when these daughters migrate, the mother completely disappears since practically all of the cells of its wall have been used in the formation of the paletots of the daughter sporocysts. One of the most striking characteristics of the mother sporocyst of the *P. muris* type is that all the daughters are at about the same stage of development. This synchronous development apparently is brought about by all the germ cells starting to develop into embryos at about the same time after the completion of a definite phase

of germ cell multiplication. Also, it seems characteristic of this type for the daughters to migrate from the mother, especially to all parts of the digestive gland of the snail. However, occasionally in mother sporocysts outside the digestive gland and more frequently in those imbedded in the gland some or all of the daughters undergo the characteristic growth stages and develop to maturity without migration, producing masses of mature daughters. It is possible to suggest that in *P. muris* the miracidium penetrates the intestine and metamorphoses on its outside into a simple sac containing a few germ cells enclosed in the layer of cells derived from the body wall of the miracidium. Then as this sac grows and the germ cells multiply, division comes about by ingrowths of the body wall, until finally each of the daughters which develop from the germ cells is surrounded by a portion of the mother sporocyst wall which forms its paletot.

A second type of mother sporocyst development was found in *A. corti*. In this case the developing daughter sporocyst embryos appear to lie in a matrix of mother sporocyst cells, from which each receives its paletot when in the round "germ ball" stage. No definite division of the mother sporocyst into compartments is present, and the embryos in a single mother vary considerably in the degree of development. The mother sporocysts in this species are always imbedded in the digestive gland of the snail, and while there was evidence of migration it appears that the daughters very frequently undergo the growth stages, which are like those of *P. muris*, without leaving the mother. We have no evidence on the method of metamorphosis of this type of mother sporocyst from the miracidium, since early stages were not found, but it is evident that the phase of multiplication of the germ cells must continue for a considerably longer time than in *P. muris* to produce the variation in the development of the embryos found in one mother sporocyst.

The development of the mother sporocyst of *P. proximus*, which is considered as the third type, appears at first glance to be entirely different from *P. muris*, since the daughters come to lie in branching tubules which form plate-like layers, and the paletots of the embryo daughter sporocysts are formed in an entirely different fashion from either *P. muris* or *A. corti*. The youngest mother sporocyst of *P. proximus* that we found (Fig. 4) gives a clue to the method of formation of the mother sporocyst of this type. It might be suggested that a simple sac containing a few germ cells is first formed by the metamorphosis of the miracidium. Simple outgrowths from this sac would early form the stage shown in Fig. 4. Continued branching of these outgrowths and elongation of the branches would produce the later stages. Characteristic of this type of mother sporocyst is the formation of plates composed of a single layer of branched tubules. At first the germ cells are massed in the tubules. Later these cells and the embryos they form become strung along the tubules. The period of germ cell multiplication must last even longer than in the development of the mother sporocyst of *A. corti* since in a single mother sporocyst all stages of development from germ cells to migrating daughter sporocysts may be present. Entirely different, also, is the method of formation of the paletots of the daughter sporocysts. As the embryos grow, that part of the tubule in which they lie comes to adhere closely to their outer wall so that when they have become elongate daughter sporocysts the tubule wall has become their outer layer or paletot. Thus are formed strings of daughter sporocysts connected by those portions of the tubules between them. When these daughter sporocysts undergo the

growth stages without migration their attachment to each other by the extensions of their paletots may still persist after they have become mature. It seems probable that when the mother sporocysts of this type develop in front of the digestive gland, the daughters usually break away from the mother and migrate to the digestive gland. They do, however, sometimes develop in mothers outside the gland without migration. As in *P. muris*, it would be expected that in mothers that develop in the tissues of the digestive gland, growth without migration would be more frequent. However, our observations on this point are very limited.

The development of the sporocysts of *M. typicus* is considered as a fourth type. In this species the daughter sporocysts are always found in front of the digestive gland. It appears that they do not migrate from the mother sporocyst and do not go through the peculiar growth stages that are characteristic of the other three types. The very immature mother sporocysts of this species are small masses of unbranched tubules attached to a common base on the outside of the bulb of the esophagus of the snails next to the stomach. Within the tubules there is a linear series of daughter sporocyst embryos, which vary from early cleavage stages to daughter sporocyst embryos with a well-developed wall and separate cercarial embryos. As indicated by Fig. 11 the paletots of the daughter sporocysts are formed by the walls of the tubules in the same way as in the mother sporocysts of *P. proximus*. Also, the variations in the developmental stages of the daughter sporocyst embryos found in a single mother indicates that in *M. typicus* there is a considerable lag in the development of the germ cells.

Although no very immature mother sporocysts were found for *M. typicus* it is possible to suggest the course of development. After the metamorphosis of the miracidium into a simple sac containing a few germ cells, there must be very extensive tubular outgrowths from the mother sporocyst wall. There is no evidence that these tubules ever form secondary branches. An extensive multiplication of germ cells must occur and these germ cells are carried along with the tubular outgrowths, and come to lie at intervals along them. As individual germ cells develop into embryos the walls of the tubule adhere to them and form the paletots. The extensive growth of the tubules and the development to maturity of the daughter sporocysts would finally form the masses of daughter sporocysts of the mature infections. A mature infection of *M. typicus*, therefore, is merely a very greatly enlarged mother sporocyst from which the daughters have never escaped. This failure to migrate from the mother means that the daughters are localized in front of the digestive gland and are never found in the tissues of this gland. It appears also that the growth of the daughter sporocysts of this species does not include the peculiar stages where protrusions are produced from areas of growth at the ends as in the other three types. The daughter sporocysts of *M. typicus* are, therefore, the smallest of any studied and contain the smallest number of cercarial embryos.

In spite of the considerable variations shown in these six species in the structure of the mother sporocyst and the development of the daughter sporocysts they all show fundamental relationships in their germ cell cycle and general development. The six species belong to two quite different families of the Plagiorchioidea, i.e., the Plagiorchiidae and the Macroderoididae. That this same general type of development occurs in a wide variety of other plagiorchiids is suggested also by the description of paletots for the daughter sporocysts of a number of different species (cf. Cort

and Olivier, 1943b). It is rather surprising, therefore, that the mother sporocysts of plagiorchids described in the literature are quite different in structure from any of the four types we have found. Three of these are of species of the Reniferidae (Talbot, 1933; Byrd, 1935; Walker, 1939) and the other is of *Plagitura parva* described by Stunkard (1936). In all these species the mother sporocysts are described as thin-walled sacs which are similar to this same stage of other digenetic trematodes. The outer coat or paletot is not shown for the daughter sporocysts of these species. It would appear, therefore, that there are wide variations in the structure of the mother sporocysts in the Plagiorchioidea, and that further knowledge of this stage may be of value in developing a more natural classification.

SUMMARY

Observations on the mother sporocysts and development of the daughter sporocysts in immature natural infections of six different plagiorchid species, *Plagiorchis muris* Tanabe, 1922, *P. micracanthos* Macy, 1931, *Cercaria talboti* McMullen, 1938, *Alloglossidium corti* (Lamont, 1921), *P. proximus* Barker, 1915, and *Macroderoides typicus* (Winfield, 1929), has given an entirely new conception of the structure and development of the sporocyst stages in this group. In all these forms the daughter sporocysts have an outer covering of cells, which forms a distinct outer layer, the paletot, which is derived from the wall of the mother sporocyst, and which persists throughout the whole life of the daughters. Characteristic also is a phase of multiplication of the cells of the germinal line, which may be interpreted as a polyembryony of the zygote, in the early development of the mother sporocyst which produces large numbers of daughter sporocysts. In the daughter sporocyst embryos after a limited period of multiplication of the cells of the germinal line, germ-masses are produced, only one for each daughter. These produce cercarial embryos throughout the whole life of the daughter sporocysts by the breaking off of multicellular components in a secondary polyembryony. This combination of extensive multiplication of cells of the germinal line, which may produce from several hundred to several thousand daughter sporocysts, with the extraordinary reproductive activity of the germ-masses in the daughter sporocysts, produces the enormous numbers of cercariae so characteristic of the plagiorchids.

Another striking point is the almost unlimited growth of the cells of the mother sporocyst wall to form the paletots of the daughter sporocyst. These paletots in some species are formed by the ingrowing of the wall of the mother sporocyst and in others from the walls of tubular outgrowths of the mother sporocysts in which the developing daughters are enclosed.

Another characteristic of the plagiorchid species studied is the growth of the daughter sporocysts after they had reached their final position in the snail host by protrusions of the wall from growth areas at the ends. This results in stages showing very bizarre shapes, which in some cases suggest multiplication by budding or fission. However, there is no evidence from our observations that this type of asexual multiplication normally occurs.

Finally, the distribution of the daughter sporocysts to the various tissues of the snail, especially the digestive gland, usually occurs by the migration of daughter sporocysts from the mother. However, not infrequently the daughters go through the growth stages and develop to maturity without leaving the mother sporocyst.

This most frequently occurs when the mother sporocysts develop in the digestive gland. In one of the species, *Macroderoides typicus*, the daughters never leave the mother and appear to develop to maturity without going through the characteristic growth stages. Whether the daughters leave the mother sporocyst or not they retain throughout their whole life as an outer layer, the paletot, a portion of the wall of the mother.

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METHODS FOR COLLECTING TICKS FOR STUDY AND DELINEATION¹

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Several years ago the author became dissatisfied with the traditional solution of 70 per cent alcohol commonly used for killing as well as preserving ticks and other arthropods. A tick killed in strong alcohol dies with its legs so contracted as to obscure important diagnostic characters. For this reason, a solution was found which killed ticks with their legs outstretched.

In addition to developing a new killing solution, several modifications of collecting techniques were evolved to meet conditions in the southern and southeastern states.

The author is indebted to the late Dr. George H. F. Nuttall, of the University of Cambridge, for suggesting the use of a tightly closed bag for carrying small vertebrate hosts parasitized by ticks. The bag as originally proposed has been modified as indicated below. To Dr. H. E. Ewing, of the U. S. National Museum, credit is due for suggesting the use of glycerine-alcohol in homeopathic, cork-stoppered vials for permanent preservation of arthropods.

METHODS OF COLLECTION

Ticks can be collected from vegetation (Kohls, 1937) by using a white cotton flannel flag which is about one yard square. The author has modified this flag by adding a strip of material so as to make two long shallow pockets down the middle of each face. A four-inch strip of cotton flannel is fastened onto the flag by means of a seam down its center. The ends of the strips are then sewn down to complete the pockets. Ticks tend to lodge in these and consequently do not fall off.

A flag seems to be relatively ineffective in the southern states where the ground is warm and ticks are less inclined to climb up into the sunshine. There ticks can sometimes be collected most effectively from the ground litter along animal trails, around dens and in bedding-down areas. At other times, the best results are obtained from walking cross-country with a large dog. The dog should be encouraged to range widely and be allowed to choose his own resting spots. Ticks will be found concentrated around the neck and ears of the dog if they are allowed time to attach. The collector's person and clothes will also be a source of ticks.

When native hosts are trapped or shot, they should be examined minutely as soon as possible after death. In order to do this, it is best to lay the carcass on a white cloth or paper so that any ticks that leave the host will be seen.

For convenience in the field, small host animals can be placed in bags as soon as killed. Preferably, the warm body should be put in a cotton flannel bag which is closed by means of a drawstring. The seam for the drawstring should contain extra material to form a ruffle within the mouth of the bag. This ruffle serves to prevent

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the escape of ticks which crawl from the chilling host. In addition, a wad of absorbent cotton should be inserted as a plug in the mouth of the bag as the draw-string is pulled tight. Most of the ticks will fall off into this bag.

Attached ticks should have their mouthparts dissected free of the host skin if wanted alive, otherwise, they should be wetted with kerosene or some other thin oil (Imes, 1926). Oiled ticks will drop off within a half hour and will die.

METHOD OF KILLING

The composition of the collecting fluid is important. Although it has been customary to collect many terrestrial arthropods into 70 per cent alcohol, the author has long preferred a solution of ninety-seven parts of 20 per cent alcohol and three parts of ether. Ticks usually spread their legs to the utmost before dying in this solution. This permits clear inspection of coxal spines and other ventral characters used in tick identification. These are usually covered by the curled-up legs of ticks which have been killed in strong alcohol.

Ticks which have been killed in this ether-alcohol solution are better for teaching purposes because their appearance is more nearly life-like and because their external anatomy is readily observed.

This technique has proven successful with ticks, hydroacarina, immature and adult aquatic insects, and many terrestrial arthropods. The container should be large enough to allow large species to die outstretched.

FINAL PRESERVATION

Since ether-alcohol is not a satisfactory preservative, the fluid should be changed as soon as possible. The author uses stoppers tinted with colored ink for his collecting vials. As soon as the fluid in the vials is replaced with the preserving solution, stoppers of the usual color are substituted.

Final preservation is in a solution containing nine parts of 75 per cent alcohol to one part of glycerine. The glycerine is insurance against desiccation in case the alcohol evaporates. Specimens are permanently stored in homeopathic vials with oversize cork stoppers rolled down to fit.

Although this method is not as desirable, all but engorged ticks can be removed from the killing solution, dried rapidly, and stored in pill boxes or other appropriate containers, between sheets of cellucotton or soft paper which has been suitably padded.

RENDERING CHITINIZED STRUCTURES VISIBLE

The presence of glycerine has one disadvantage. Clear, chitinized structures, such as the coxal spines and the teeth of the hypostome and of the chelicerae of ticks become almost invisible. For detailed examination of these parts the ticks must be washed repeatedly in fresh 70 per cent alcohol and viewed by lateral illumination. If the parts are still hard to see, they can be stained with dilute aniline dyes, such as methylene blue. A more satisfactory staining is obtained by using Galigher's modification of Bethe's method for testing for chitin (Galigher, 1934), which is as follows:

MODIFIED BETHE'S METHOD FOR STAINING CHITIN

1. Transfer the tick from clean, glycerine-free alcohol to a solution containing 95 cc. of 90 per cent alcohol and 5 cc. of concentrated nitric acid. Material should remain in this mixture—3–5 days.

2. Wash in three changes of 50 per cent alcohol.
 3. Place ticks for three minutes in freshly prepared 10 per cent aqueous solution of aniline hydrochloride, to which one drop of hydrochloric acid has been added to each 10 cc. of solution.
 4. Rinse in water.
 5. Place in 10 per cent aqueous solution of potassium bichromate until stained an intense grass green.
 6. Rinse in water.
 7. Place in water containing one drop of ammonium hydroxide per every 10 cc. The color will soon change to blue.
 8. Wash in water for five minutes and return the specimen to 70 per cent alcohol.
- Note:—Steps 3 and 5 may be shortened or protracted according to the size of the structures to be stained. The parts should be stained a clear, semi-transparent blue.

SUMMARY

1. Methods are described for collecting ticks from soil, vegetation and host animals.
2. It is important for diagnostic purposes that ticks be killed in an expanded condition. The author has found that a solution of 3 parts of ether and 97 parts of 20 per cent ethyl alcohol will kill ticks with their appendages expanded.
3. This ether-alcohol solution has proven useful in killing many different arthropods, both aquatic and terrestrial.
4. Preferably ticks and other arthropods should be transferred to a solution of one part glycerine and nine parts of 75 per cent alcohol for final storage, however, the more highly chitinized species may be dried.
5. An outline of a modified Bethe's method for staining chitin is suggested for studies of small, clear, chitinized structures.

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EIMERIA BREVOORTIANA, A NEW SPOROZOAN PARASITE
FROM MENHADEN (*BREVOORTIA TYRANNUS*), WITH
OBSERVATIONS ON ITS LIFE HISTORY

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INTRODUCTION

Most of the species of the genus *Eimeria* are intestinal parasites throughout their life histories. In a few instances oöcysts have been found in various other organs of the host animal, such as kidney, spleen, liver, air bladder and testis. With the exception of one, *Eimeria stiedae* (Lindemann), which is parasitic in the liver of rabbits, the life histories of these species are incompletely known. Any information, therefore, which helps explain their peculiar behavior will no doubt be of value in determining the relationships of other members of the genus and of the genus itself.

During the course of an investigation of the parasites of marine fishes, oöcysts of an eimerian parasite were found in the testes of menhaden. Two parasites of this nature have previously been described from the testes of fishes, *E. sardinae* (Thélohan, 1890) from sardines, and *E. nishin* Fujita (1934) from herring. In these cases, as with the present species, the only stages seen in the testes were those that belonged to the sporogonic cycle, zygotes, sporocysts, and sporozoites.

Since the typical life history of an eimerian includes a schizogonic as well as a sporogonic cycle, a study was begun to determine the location of the former in menhaden. Smears and sections of testes, stomach, pyloric ceca, intestine, spleen, liver and kidney were examined. Eventually, structures resembling the schizogonic stages of other eimerians were found in the epithelium of the pyloric ceca—spores, schizonts, macro- and microgametocytes. Together with the cycle of sporogony in the testes they form a complete life history of an eimerian, which is described in the following pages.

The writer is indebted to many friends for help during the course of the work, particularly to Dr. A. S. Pearse of Duke University, under whose direction it was carried out. Dr. H. F. Prytherch kindly extended the facilities of the United States Fish and Wildlife Service Laboratory at Beaufort, North Carolina. Capt. Chas. Hatsell and many fishermen of Beaufort helped in collecting material, and Margaret Hardcastle aided the writer greatly in the examination of much of it. Dr. D. C. Boughton of the United States Bureau of Animal Industry read the manuscript and made many helpful suggestions.

MATERIALS AND METHODS

All material was collected at Beaufort, North Carolina, during the years 1937–1942. Collections included specimens from various parts of the coast, at all seasons of the year.

During the early part of the work it was found essential that material for the

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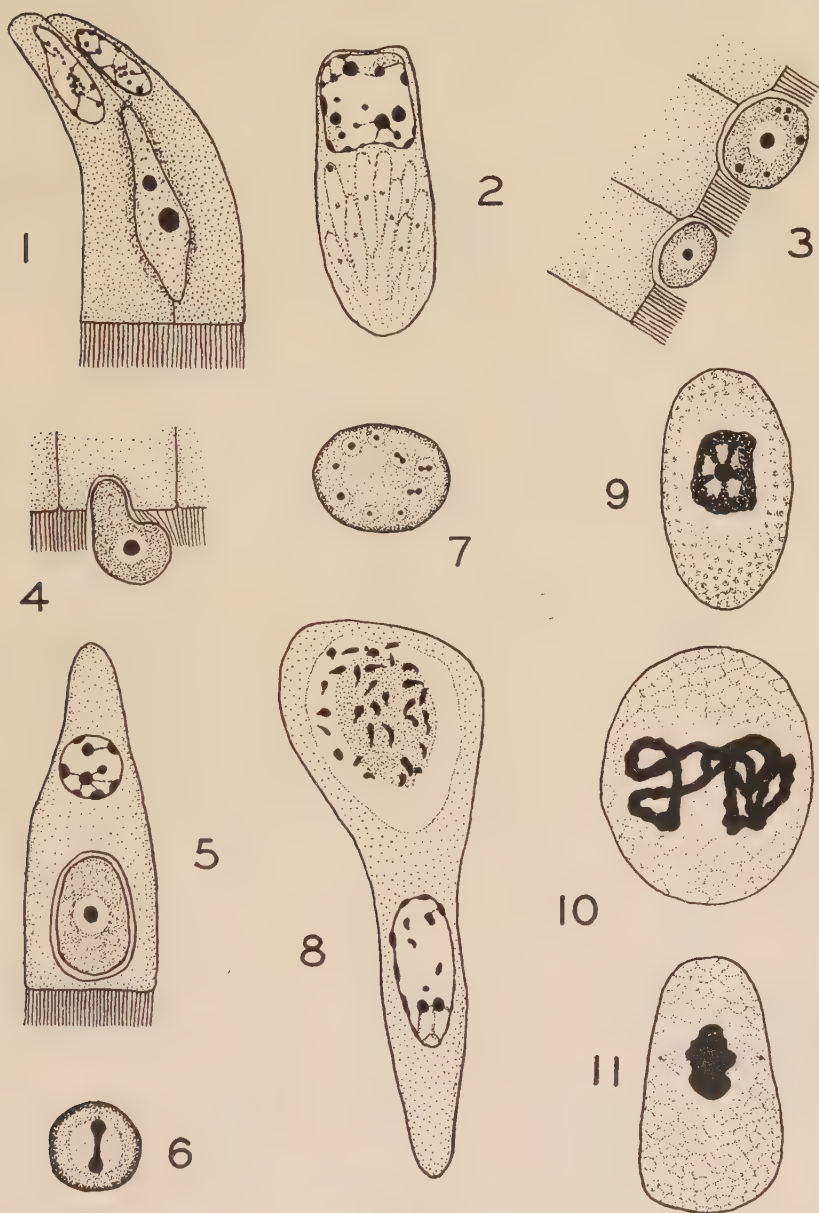


FIG. 1. Developing trophozoite in epithelium of ceca.
 FIG. 2. Mature schizont.
 FIG. 3. Young gametocyte in ciliated border of cecal epithelium.
 FIG. 4. Young gametocyte penetrating epithelial cell.
 FIG. 5. Intracellular gametocyte.
 FIGS. 6-7. Division of nucleus of microgametocyte.
 FIG. 8. Nearly mature microgametocyte in host cell.
 FIG. 9. Macrogametocyte.
 FIGS. 10-11. Division of macrogamete or zygote.

study of intracellular stages be absolutely fresh. Since this necessitated fixation of tissue in the field, Bouin's fluid was used almost exclusively, with satisfactory results. Tissues were embedded and sectioned in the usual manner. Schizogonic stages in the ceca were stained with Heidenhain's iron hematoxylin and counterstained with light green. Ehrlich's hematoxylin gave best results with testicular tissue.

Fresh oöcysts were obtained by the same methods generally used to collect them from feces. Portions of testis were thoroughly pulverized in a little water, strained through cheesecloth, centrifuged, and the oöcysts floated with a concentrated salt solution. All measurements were made in a hanging drop suspended over a depression slide.

For the study of sporocysts and sporozoites, oöcysts were crushed between a coverslip and slide. The resulting smear was fixed in Schaudinn's fluid and stained with Ehrlich's hematoxylin.

All drawings were made with the aid of a camera lucida. Oöcysts were drawn from fresh material to an original magnification of $660\times$; all other drawings were made from stained sections or smears to an original magnification of $1350\times$.

LIFE HISTORY

Trophozoites

The earliest stages of the parasite seen in the ceca were trophozoites that had penetrated the epithelium lining (Fig. 1). These were rarely seen, and only in isolated groups. They are cigar-shaped structures measuring about 12 microns in length by about 2.5 microns in width. In the center is a large nucleus, which in the early stages is a round solid mass of chromatin. At the anterior end is a mass similar to, but smaller than, the nucleus, presumably a refractive globule. In most of the trophozoites a bulge is present near the center, so that hardly any two of them are of the same size and shape. It seems likely that they develop rapidly after entering cells.

Schizonts

As the schizont enlarges the nucleus divides into small fragments which separate. Hazy lines appear in the cytoplasm and each encloses a fragment of the nucleus to form a merozoite.

Mature merozoites measure 3 to 5 microns in length by 0.5 to 1 micron in width. Usually 8 to 10 are present in a single schizont. Their anterior end is slightly enlarged and the other tapers to a slender tail which extends toward the outer end of the cell. In the center a nucleus is barely visible.

Mature schizonts (Fig. 2) average 12 microns in length by 6 microns in width, although their size is extremely variable. They may be compressed into long slender structures, or be shortened and almost round, depending probably on the pressure of surrounding cells.

Nothing definite can be said about the number of generations of schizonts. The merozoites could not be measured accurately enough to detect differences in size, and smears from fresh material were not successful. However, the fact that schizonts are by far the most commonly seen stage would seem to indicate that more than one generation is produced.

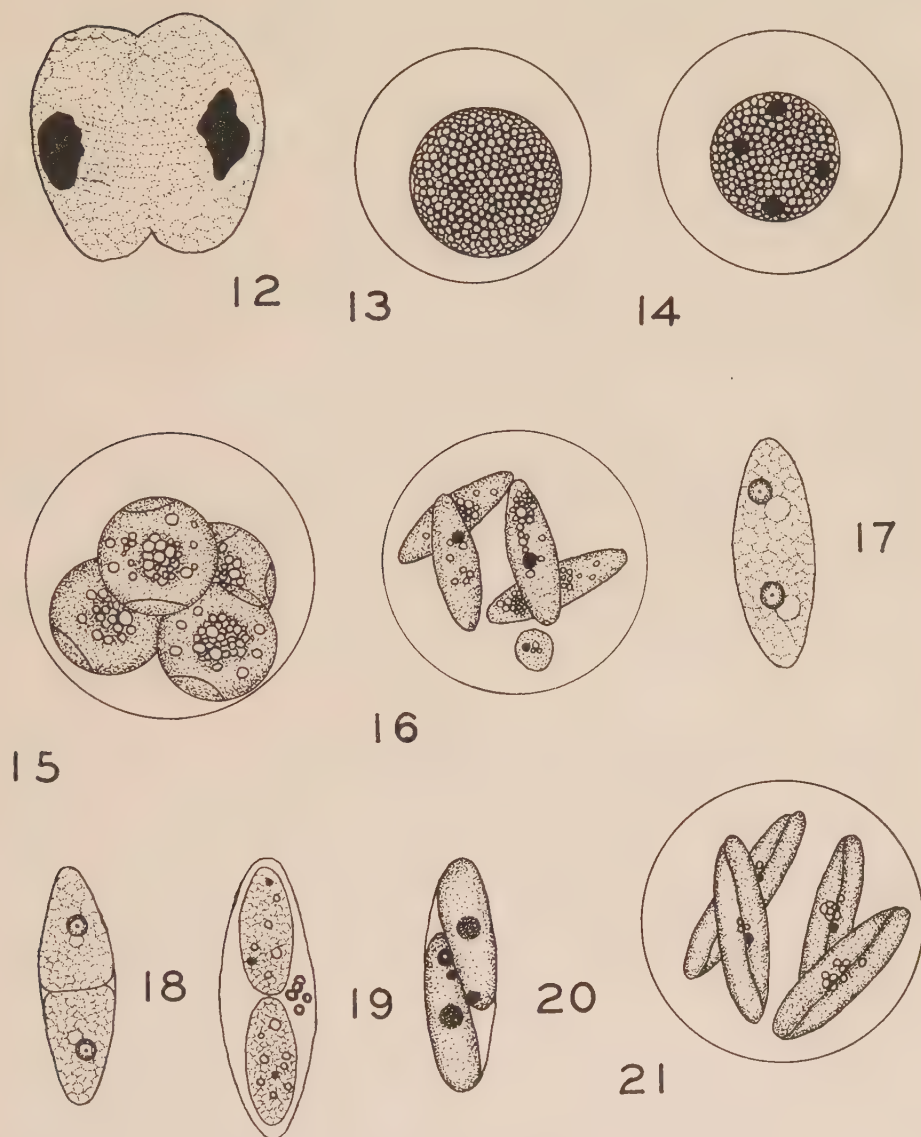


FIG. 12. Later stage in division of macrogamete or zygote.

FIG. 13. Oöcyst containing undifferentiated zygote.

FIG. 14. Division of zygote nucleus.

FIG. 15. Oöcyst containing sporoblasts.

FIG. 16. Oöcyst containing spores.

FIGS. 17-20. Formation of sporozoites.

FIG. 21. Fully sporulated oöcyst.

Microgametocytes

Since the merozoites are of such small size, the earliest gametocytes are barely visible under a magnification of 1250. They are almost invariably found lodged in the ciliated border of the epithelial cells (Fig. 3). Possibly some of the merozoites themselves penetrate cells, but gametocytes usually found there (Fig. 5) are in a rather advanced state of development. Their appearance is typical. Each consists of a granular cytoplasm that takes a fairly heavy stain, and surrounds a perfectly clear area. In the center of this area is a small, round, heavily staining mass of chromatin. The young gametocytes of both sexes are identical, as far as could be determined. The cytoplasm is essentially similar and granules may be found scattered through each.

The gametocytes generally develop in the ciliated border of the host's intestinal epithelium until a diameter of about 5 microns is reached. From this size they may continue their development extracellularly, but the majority bore into adjacent cells, apparently by ameboid movement (Fig. 4). The significance of this behavior will be discussed later.

After entering a cell, development follows the usual procedure. The nucleus divides and subdivides until the whole structure is filled with daughter nuclei. Each then begins to elongate, finally reaching a length of about 2 microns (Figs. 6 and 7). Flagella have not been seen but it is assumed that they are present.

When maturity is reached, the microgametocyte is about 12 microns in diameter and fills the outer half of the host cell (Fig. 8). The microgametes are scattered about over the exterior, and as they leave, the greater part of the microgametocyte remains as a residual mass in the cell.

Macrogametocytes

Macrogametocytes can be identified by the time they have reached a diameter of 5 microns. They greatly outnumber the microgametocytes, in fact, with the exception of schizonts, they are the most common stage encountered.

Macrogametocytes also can probably complete their development in the ciliated border of the cecal epithelium, but seldom do so. When they enter a cell they barely get inside and take up a position between the nucleus and the outer border; only in rare instances have they, or any stage, been seen in the basal portion. In the cell they continue to enlarge until a diameter of about 10 microns is reached. At this time the macrogametocyte undergoes a reorganization. The cytoplasm becomes more homogeneous, the clear area about the nucleus disappears (Fig. 9), and eventually a complete division takes place (Figs. 10-12), resulting in two daughter cells.

These events are not at all clear, due in part to the impossibility of getting a complete series of stages, and in part to the fact that no information could be obtained as to when fertilization occurs. It seems probable that the macrogamete is fertilized just before, or during, its reorganization. If such is true, the division may be a reduction division which involves the whole zygote, resulting in two migratory post-zygotic stages, or ookinetes, which will carry the infection to the testes.

Oöcysts

All of the stages described thus far occur in the host's pyloric ceca, while those that follow are in the testes. It will be assumed for the present that the second cycle follows immediately after the first, and discussion of this point will be reserved until later.

The earliest stage seen in the testes is a typical oöcyst, which consists of a fully formed cyst wall that encloses a granular protoplasmic mass (Fig. 13). Some of the oöcysts are very much smaller (12 microns) than a fully sporulated oöcyst (25 microns), and the enclosed mass completely fills the interior. About 700 testes, in all stages of development have been searched for an earlier stage than this, but nothing has been seen which in any way might be so interpreted.

In some species of *Eimeria* it is believed that the protoplasm shrinks away from the cyst wall during development, creating a space between. In this species the size of the protoplasmic mass apparently remains about the same; those measured varied from 15 to 19 microns in diameter, with an average diameter of 15.7 microns in both large and small oöcysts. The average diameter of the oöcysts that contain fully developed zygotes is 22.59 microns, compared to an average diameter of 25.09 microns for fully sporulated oöcysts. This appears to indicate that enlargement of the cyst wall, rather than shrinkage of the protoplasm, takes place.

In the fresh material the central mass is composed of small indistinct granules of stored food, enclosed by a membrane. No nucleus is visible. In stained sections the granules are quite distinct, and present a rather loose arrangement. The nucleus appears as a dark central area, and usually is in some stage of division. This apparently is a lengthy process, as zygotes just on the verge of division have been kept under observation for 12 hours without any sign of change.

The first division results in two nuclei which take up positions on opposite sides of the zygote. These in turn divide and produce four nuclei which migrate to the periphery to form the corners of a square (Fig. 14). The cytoplasm then divides to produce four sporoblasts and a residual body.

The sporoblasts (Fig. 15) are circular and measure about 10 microns in diameter. Each has its own share of food material and a portion of chromatin. The latter, in most cases, does not seem to reorganize but immediately begins the nuclear division that will form the sporozoites.

Fully mature spores (Fig. 16) average 16.4 microns in length by 6.3 microns in width. In fresh material they appear to be filled with granules of various sizes, similar to the undifferentiated zygote. In stained sections they show a slightly granular cytoplasm with two nuclei, one at each end, and a vacuole close by each (Fig. 17). A karyosome is present in each nucleus.

The division of the spore into sporozoites is quite interesting. The spore elongates somewhat and the protoplasm begins to pinch in two transversely at the center (Fig. 18). This occurs inside the sporocyst, which does not itself divide. The protoplasm finally becomes completely divided and each half begins to pull apart and to separate from the sporocyst, producing two oval bodies which rest end to end (Fig. 19). A small sporocystic residuum is also formed during the division. The oval sporozoites now begin to elongate and grow more slender, eventually filling the sporocyst (Fig. 20). They are cigar-shaped, slightly curved structures with one end pointed and the other blunt. A large nucleus is present in the center. When mature (Fig. 21) they average 15.0 microns in length by 2.5 microns in width. In the sporocyst they lie side by side, slightly curved about each other, and face in opposite directions. With completion of this process the oöcyst is fully formed and ready to infect a new host.

The oöcysts, like those of many other species, occur in both spherical and oval

shapes. The round type is by far the most common, and ranges in diameter from 17.50 to 30.00 microns, with a mean diameter of 25.09 microns. Range in size of the oval oöcysts is $21.20\text{--}30.00 \times 15.00\text{--}27.50$ microns, with a mean size of 26.16×22.73 .

Of the 700 fishes examined, 41.9 per cent of the males were infected. This figure is lowered considerably by the infrequency of infection in younger fishes; infection often runs as high as 100 per cent in a large group of mature fishes. No parasites were ever seen in the ovaries of females, although the schizogonous stages occur in the ceca. They either do not continue their development or it occurs in some other part of the body.

DIAGNOSIS

Eimeria brevoortiana n. sp., parasitic in the testes of menhaden, *Brevoortia tyrannus* (Latrobe). Oöcysts occur in round and oval forms. From 300 measurements the range in diameter of round oöcysts was 17.50–30.00 microns, with a mean diameter of 25.09 microns; range in size of oval oöcysts, $21.20\text{--}30.00 \times 15.00\text{--}27.50$ microns, with a mean size of 26.16×22.73 . Oöcystic and sporocystic residua present. No micropyle present. *E. brevoortiana* is differentiated from *E. sardinae* and *E. nishin*, also parasitic in the testes of fishes, by the much greater size of the latter (45–50 microns).

DISCUSSION

The life history of *Eimeria brevoortiana*, here set forth, exhibits one fundamental difference from that described for other members of this genus—schizogony and sporogony occur in different organs of the host. This difference, at first consideration, appears to be a radical departure from the usual procedure in which sporogony follows schizogony in the gut without interruption. Such a life history as the one described, however, might well be expected when certain species with similar characteristics are considered. *E. truncata* (Railliet and Lucet) parasitizes the kidneys of geese, *E. gadi* Fiebiger the swimbladder of cod fish, *E. kermoganti* (Simond) the spleen of gavials, and *E. sardinae* (Thélohan) and *E. nishin* Fujita the testes of fishes. The way in which these errant coccidians gain access to the organs they parasitize is not yet known, so it seems quite certain that some members assigned to this genus have life histories that do not conform to the types now known.

In the present case, the fact that stages typical of an eimerian parasite have been found in the testes, suggests that schizogony occurs at least in the same host, if not in the same organ. Seven hundred testes have been examined, at all seasons of the year and in all stages of development, and there is no evidence whatever to indicate that any stages of schizogony occur in them. As mentioned previously, other organs likely to harbor such stages have been examined with negative results. Since the stages in the gut are typical and are the ones required, they are believed to be parts of the life history of the same parasite.

On the other hand, the schizogonic phase found in the pyloric ceca would normally be followed by a sporogonic phase, in which oöcysts would be produced and liberated in the feces. To test this possibility, the intestinal contents of 170 fishes were examined at all seasons of the year but no oöcysts were found. This is strong evidence that such are not present.

Ordinarily a question such as this could be answered conclusively by feeding infective stages to parasite-free hosts and tracing their development. In the case of

menhaden, however, their fragile nature, and the impossibility of securing parasite-free specimens precluded such experiments.

As to the means by which the parasite reaches the testes, there are three possibilities—the blood stream, through the cloaca, or penetration of the intervening tissues. Regarding the first, Smetana (1933) has demonstrated that the sporozoites of *E. stiedae* (Lindemann) are hatched in the gut of rabbits and that they bore through the intestinal walls and reach the liver by way of the portal veins. The fact that one member of the genus *Eimeria* employs the blood stream as a means of transportation suggests that *E. brevoortiana* may use the same method.

Halawani (1930) found oöcysts of *E. southwelli* in the spiral valve of intrauterine devil-fish embryos, and suggested that the parasite had traveled down to the cloaca of the mother and ascended the uterus. The vasa deferentia of many menhaden were examined with this in mind, but no zygotes or earlier stages were ever found there.

It seems more likely that the testes may be reached by active migration through the tissues. As previously mentioned, the early gametocytes begin their development in the ciliated border of the epithelium and later appear to bore into a cell, as illustrated in Fig. 4. This behavior is quite interesting. In some species such as *E. gadi* Fiebiger (1913) and *E. mitraria* (Laveran and Mesnil, 1902), the entire development of the parasite takes place extracellularly. In contrast to these, the great majority of eimerians are intracellular parasites. There is another group, however, to which *E. brevoortiana* belongs, which exhibits a transition from one type to the other. *E. anguillae* Léger and Holland (1922) is extracellular in development, but when the macrogametocyte matures it bores in to the base of the epithelium and there sporulates. *E. pigra* Léger and Bory (1932) is extracellular as a rule, but occasionally certain of the stages enter cells. Léger and Bory regard this as the beginning of an adaptation to intracellular life.

The fact that the early gametocytes of *E. brevoortiana* are motile, suggests the possibility that they may be motile at other stages as well. This seems plausible in light of the observations of Elmassian (1909) on the macrogametocytes of *E. rouxi*. He writes—"le *Coccidium* (= *Eimeria*) *rouxi* est essentiellement migrateur et cela toutes les périodes et toutes les phases de sa vie, même quand il revêt la forme de cellule sexuelle. . . . Cela surtout est vrai pour les gamètes qui avant d'atteindre le terme de leur développement ont besoin de se rechercher en vue de la fécondation." He states that he has actually seen the macrogametocytes boring through the epithelium of the host. In consideration of these facts, and observations on the present species, it is believed that in the case of *E. brevoortiana*, infection is carried from the gut to the testes by a motile macrogamete or zygote by active penetration of the tissues.

The question also arises as to how and when infection takes place. Spawning of menhaden, from the meager accounts available, takes place off shore, where schools of fish congregate and shed their eggs and milt into the water. In a few mature males which have been examined, sporulated oöcysts were found in the vasa deferentia and mixed with the sperm stripped from the testes. If they are passed into the water in this way, they could easily be picked up by other hosts, since menhaden are plankton feeders and have numerous long gill rakers which serve as a very efficient straining apparatus.

SUMMARY

A new species of sporozoan parasite, *Eimeria brevoortiana*, has been described from the menhaden, *Brevoortiana tyrannus* (Latrobe). All stages of sporogony were seen in the testes and these are described. No evidence of schizogony was seen in 700 testes examined.

All stages of schizogony characteristic of members of the genus *Eimeria* were found in the epithelium of the pyloric ceca of menhaden and these are described. No such stages could be found in other organs of the host. The intestinal contents of 170 menhaden were examined but no oöcysts were found.

From the evidence at hand it appears logical to assume (1) that the two cycles are continuous, and are parts of the life history of the same parasite; (2) that infection of the gut is carried to the testes of the same host by a migratory stage; (3) that infection of other hosts takes place by oral ingestion of oöcysts which have passed into the water with sperm from infected hosts.

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ACQUIRED RESISTANCE IN CHICKENS, TURKEYS, AND RING-NECKED PHEASANTS TO THE GAPEWORM, *SYNGAMUS TRACHEA*

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A number of investigators have reported observations on the resistance of chickens to the poultry gapeworm, *Syngamus trachea*. Ransom (1921) showed experimentally that as chickens grow older they become increasingly resistant to gapeworm infection. Clapham (1934) reported that 13-week-old chickens are refractory to infection, while Waite (1920) was successful in infecting two of three yearling hens by feeding them large numbers of earthworms infected with the larval forms of these gapeworms. Ransom (1921) infected 5 of 17 mature chickens to which large numbers of embryonated eggs and larvae of this nematode had been previously given. Morgan (1931) found one yearling hen infected with 12 pairs of worms, and Crawford (1940) observed that it was not unusual to find adult chickens infected with gapeworms in Ceylon. The latter investigator stated that "the number of pairs of worms found in the tracheas of adult fowls is generally small, as a rule not more than two or three pairs, but not infrequently adult hens have been seen on poultry farms exhibiting typical clinical symptoms. . . ." Olivier (1943) observed that 7 of a flock of 13 mature chickens were infected with gapeworms following exposure to contaminated soil at the Beltsville Research Center, Beltsville, Md. One of these birds showed typical symptoms and harbored 94 pairs of worms at necropsy.

Clapham (1934) concluded that diets low in vitamin A or in minerals, particularly calcium, reduced the resistance of 13-week-old chickens to *Syngamus*.

Whitlock (1937) observed that female partridges were more susceptible to *Syngamus* infection than the male birds. Clapham (1939) also observed this sex difference in susceptibility of partridges and noted that the young birds had heavier infections than the older birds, although the latter were not immune.

No account of investigations on acquired resistance to *Syngamus* in turkeys and pheasants appears to have been reported other than an abstract (Olivier, 1942) of some of the material contained in this paper. It is generally considered that an intimate association between host and parasite is necessary for the induction of an immune response in the host. The larvae of *S. trachea* penetrate the tissues of the host and also live for a time in the lungs. As adults they are attached to the tracheal mucosa. Therefore, it was suspected that an acquired resistance to this parasite might be induced in susceptible birds, and the six experiments described in this paper were undertaken to investigate that possibility.

MATERIALS AND METHODS

All the birds were obtained as one-day-old chicks, and were held under parasite-free conditions until used in the experiments. The Rhode Island Red chickens and the White Holland, Bronze, and Beltsville Small White turkeys were supplied by the Animal Husbandry Division, Beltsville Research Center, Beltsville, Md. The

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pheasants were obtained from the Loyalsock State Game Farm, Loyalsock, Pennsylvania, through the kindness of Mr. Richard Gerstell, Chief, Division of Propagation and Research, Pennsylvania State Game Commission.

Material for inoculation of birds was prepared by teasing apart in water the uteri of mature gapeworms taken from turkeys. The cultures were held at room temperature until the eggs had embryonated. Soon after becoming embryonated, many of the eggs hatched. Under refrigeration the freed larvae remained active and infective for several weeks. Consequently, the inoculating material consisted of both embryonated eggs and hatched larvae, usually in the ratio of about two of the former to one of the latter.

The infective material for each of the experiments was obtained by mixing several individual cultures, none of which was over three months old. The concentration of eggs and larvae was determined by counts of a 0.15-cc culture sample. The measured dose of eggs and larvae was introduced into the esophagus of the bird near the crop by means of a long, calibrated pipette.

Four to six weeks following the first administration of infective material, a second dose of eggs and larvae was given to determine whether the birds had acquired resistance. The birds were autopsied two weeks after the second inoculation. This time interval was selected in order (1) to allow sufficient time for the worms of the second inoculation, if any, to develop and reach the trachea and to establish themselves there, and (2) to make it easier to differentiate, on the basis of size and degree of development, between the worms of the two inoculations. Observations showed the worms of the second inoculation were much smaller and the males less firmly attached to the tracheal wall; their heads were not embedded in a nodule as in the case of the males developing from the first infection.

EXPERIMENTAL DATA

Turkeys

Experiment I (see Table 1).—Each of 20 turkeys, seven weeks old, was given 10,000 embryonated eggs and larvae of *Syngamus* on June 24. Five of these birds died between the 19th and 25th days after inoculation, and, at necropsy, they harbored an average of 235 pairs of worms per bird. Groups I and III in the table represent the 15 surviving birds. Thirty-seven days¹ after the infective material was administered the birds of group III were autopsied to determine the approximate number of worms per bird prior to administration of the second dose. An average of 22.6 pairs of worms per bird was found in the seven birds. On August 4 the birds of group I (previously infected) and eight uninfected birds of the same age (group II) were each fed 5,000 eggs and larvae to determine their comparative resistance. At autopsy 15 days later, group I birds had an average of 2.3 pairs of worms of the first infection but harbored no worms of the test infection, while group II birds harbored an average of 107.9 pairs of worms per bird.

Experiment II (see Table 1).—Three groups (I, II and III) of turkeys, 40 days old, were fed 2,500 embryonated eggs and larvae per bird on September 17; group IV was held as controls. The birds of group I were autopsied two weeks after inoculation, and the birds of group II were autopsied four weeks after inoculation. An average of 24.1 pairs of worms per bird was recovered from group I and an average of

¹ In a previous report (Olivier, 1942) this figure was erroneously given as 41 days.

19.3 pairs of worms per bird was recovered from group II. On October 15, the birds of group III (previously inoculated) and group IV (uninfected) were each given 5,000 eggs and larvae. Two weeks later the birds of both of these groups were autopsied; those of group III harbored an average of 15 pairs of worms per bird of the original infection and an average of 2.1 pairs per bird of the test infection. How-

TABLE 1.—Results of experiments on resistance of young turkeys, ring-necked pheasants, and chickens to infection with *Syngamus trachea*

Group No.	No. of birds	First inoculation of eggs and larvae (number)	Av. No. worms per bird two weeks after inoculation (pairs)	Av. No. worms per bird four weeks after inoculation (pairs)	Total No. worms at autopsy	Test inoculation of eggs and larvae (number)	Pairs of worms per bird at autopsy	
							Av. No. first infection	Av. No. second infection
Experiment I (Young turkeys)								
I	8	10,000	...	...		5,000	2.3	0
II	8		...	...		5,000	...	107.9
III	7	10,000	...	22.6			...	
Experiment II (Young turkeys)								
I	16	2,500	24.1	...			...	
II	16	2,500	...	19.3			...	
III	16	2,500	...	...		5,000	15.0	2.1
IV	15		...	...		5,000	...	54.4
Experiment III (Ring-necked pheasants)								
I	17	3,000	...	...		5,300	0.0	0.7
II	5		...	...		5,300	...	94.0
III	5	3,000	...	...	One dead pair. Two living males		...	
Experiment IV (Chickens)								
I	12	2,500	7.6	...			...	
II	12	2,500	...	0.7			...	
III	11	2,500	...	...		5,000	0.2	0.0
IV	12		...	...		5,000	...	0.7
Experiment V (Chickens)								
I	24	2,100	10.1	...			...	
II	24	2,100	...	0.9			...	
III	24	2,100	...	...		3,000	0.45	0.0
IV	24		...	...		3,000	...	5.4
Experiment VI (Chickens)								
I	24	1,500	10.8	...			...	
II	24	1,500	...	5.6			...	
III	24	1,500	...	...		4,100	0.6	3.5*
IV	24		...	...		4,100	...	3.3

* Twelve of the 13 birds harbored no worms of the second infection. The remaining bird harbored 45 pairs.

ever, no worms of the second infection were recovered from 11 of the 16 birds of this group. On the other hand, the birds of group IV harbored an average of 54.4 pairs of worms per bird of the second infection, all the birds being infected. The difference in the number of worms of the second infection recovered from the two groups is highly significant, since the difference between the means is 5.4 times its standard error.

The results of these two experiments show clearly that turkeys developed a marked resistance to infection with *S. trachea* as a result of a single previous infection. In the first experiment the previously infected birds were negative at necropsy for worms of the test infection while the uninfected controls were heavily infected. In the second experiment the previously infected birds acquired only a few worms of the test infection while the controls became heavily infected. In the birds of both experiments worms of the immunizing infection were present when the test dose was administered.

Ring-necked Pheasants

Experiment III (see Table 1).—Each of fifty pheasants, 39 days old, was fed 3,000 embryonated eggs and hatched larvae on July 14. Twenty-eight of these birds died between the 13th and 27th days after inoculation, and an average of 74 pairs of gapeworms per bird was recovered at necropsy. The 17 birds of group I and the five birds of group III listed in table I represent the survivors. The birds of group II were treated like those of the other two groups except that they were not given the immunizing dose. Forty-one days after inoculation the birds of group III were autopsied and only one dead pair and two living males from which the females had become detached and lost were recovered. The following day each of the birds of group I and II was fed 5,300 eggs and larvae. At autopsy 13 days later, 12 worms, all of the second infection, were recovered from 7 of the 17 birds of group I; 10 of the birds of this group harbored no worms. The controls, however, were all infected, harboring 470 pairs of worms of the second infection, or an average of 94 pairs per bird. This difference is 6.3 times its standard error.

The results of this experiment demonstrate that pheasants developed a marked resistance as a result of heavy infections with *S. trachea*. At autopsy, the previously infected birds had no worms, or only very few, following the second dose. On the other hand, the uninfected birds became heavily infected following the second dose. Since the birds of group III had lost almost all of their worms as revealed by a post-mortem examination on August 24, it is probable that the birds of group I were almost worm-free when given the second dose on August 25. Nevertheless, they showed a high degree of resistance to reinfection. It appears, therefore, that the resistance developed by these birds persisted after the worms which induced the resistance were lost.

Chickens

Experiment IV (see Table 1).—Two groups (I and II) of 12 birds each and one group (III) of 11 chickens, 45 days old, were each given 2,500 embryonated eggs and hatched larvae on September 17. Another lot of 12 birds (group IV) was held as controls. At necropsy two weeks after inoculation the birds of group I harbored an average of 7.6 pairs of worms each; four birds had no worms at all. Four weeks after inoculation the birds of group II harbored an average of 0.7 worm per bird; eight of these birds had no worms. On October 15, each of the birds of groups III and IV was fed 5,000 eggs and larvae. Two weeks later the birds of group III harbored a total of two pairs of worms of the first infection and no worms of the second infection. The controls harbored eight pairs of the second infection, or an average of 0.7 pair per bird. Eight of the birds in this group had no worms.

Experiment V (see Table 1).—Ninety-six chickens, 21 days old, were divided

into four groups. The birds of groups I, II and III were each fed 2,100 embryonated eggs and hatched larvae on December 27. Those of group IV were held as controls. The birds of group I, when autopsied two weeks later, harbored an average of 10.1 pairs of worms per bird; six of the birds had no worms. An average of 0.9 pair of worms per bird was found in the birds of group II when autopsied four weeks after inoculation; eight of these birds had no worms. On January 27, the remaining birds of group III (11 died following the first inoculation) and each of group IV was fed 3,000 eggs and larvae. Twelve days later both groups were autopsied. The birds of group III harbored an average of 0.5 pair of worms of the original infection and none of the second infection. The controls harbored an average of 5.4 pairs of worms per bird; three of them had no worms.

Experiment VI (see Table 1).—Ninety-six chickens, 21 days old, were divided into four groups. The birds of group I, II and III were each fed 1,500 embryonated eggs and hatched larvae on January 27. Those of group IV served as controls. The birds of group I were autopsied on February 11 and were found to harbor an average of 10.8 pairs of worms per bird; one of the birds had no worms. An average of 5.6 pairs of worms per bird was found in the birds of group II when autopsied four weeks after inoculation; two of these birds had no worms. On February 24, the remaining birds of group III and each of group IV were fed 4,100 eggs and larvae. Fourteen days later both of these groups were autopsied. The birds of group III harbored a total of 8 pairs of worms of the original infection and 45 pairs of worms of the second infection. However, the 45 pairs of worms of the second infection were found in one bird, which also harbored three of the eight pairs of the original infection. Therefore, it may be readily assumed that some factor or factors must have entered in to break down the resistance or immunity in this bird, since none of the other birds harbored worms of the second infection. This same bird likewise harbored the largest number of pairs of worms of the first infection of any single bird. Twelve of the 22 control birds were infected and none harbored more than 21 pairs of worms.

DISCUSSION

An analysis of the data of the foregoing experiments has revealed some interesting facts in addition to those which have a direct bearing on the problem concerned in this paper. It affords, first of all, an opportunity to compare infections in chickens and turkeys of the same age to which were fed similar dosages of infectious material. Each of the birds of groups III of experiments II and IV received an initial dose of approximately 2,500 eggs and larvae. Four weeks later, the surviving birds of each of these groups were each given a second dose of approximately 5,000 eggs and larvae. At necropsy 14 days later, the turkeys were found to have retained more worms of both the first and second infections than did the chickens, thus indicating that the turkey is a more suitable host for this parasite than is the chicken.

The data from group I of experiments II and IV afford another opportunity to compare infections in chickens with those in turkeys. These two groups were tested concurrently and eggs and larvae from the same cultures were used to infect both groups. Both were necropsied 14 days after infection. The results indicate that the turkeys were more susceptible to infection with gapeworms since they harbored three times as many worms as the chickens.

The data from group III of experiments II, III and IV offer a basis for com-

paring the fate of *S. trachea* adults in the three species of hosts when the birds were held for 6 weeks after infection and then autopsied. At that time 1.2 per cent of the worms introduced were recovered from the turkeys, none from the pheasants, and 0.02 per cent from the chickens. It appears, therefore, that pheasants tend to lose their infections more rapidly than turkeys.

It is of particular interest to note also that in every case where comparison could be made, the percentage development of the worms varied inversely with the number of infective eggs and larvae fed. Those groups which involved birds of the same species of about the same age and which were necropsied at about the same time after infections were considered comparable groups. For instance, group III of experiment I and group III of experiment II, both involving turkeys, are comparable. The former group was fed 10,000 eggs and larvae per bird and the latter, 2,500 eggs and larvae per bird. At autopsy, those birds to which the larger doses had been given had fewer than half as many worms as those which had received the smaller doses. Data for these two groups and for four sets of comparable groups involving chickens are presented in Table 2. The differences in percentage development in the groups

TABLE 2.—Data from five comparable sets of birds to show relation between the percentage development of *Syngamus trachea* and the number of eggs and larvae administered

Experiment number	Group number	Number of eggs and larvae fed	Age of bird when fed	No. of days worms were in bird	Percentage development	No. of worms per bird
I	III	10,000	49	37	0.5	22.6
II	III	2,500	41	42	1.2	15.0
V	I	2,100	21	14	1.0	10.1
VI	I	1,500	22	15	1.4	10.8
IV	I	2,500	45	14	0.6	7.6
V	IV	3,000	50	12	0.4	5.4
VI	IV	4,100	50	14	0.2	3.3
V	II	2,100	21	28	0.09	0.9
VI	II	1,500	22	28	0.7	5.6
V	III	2,100	21	41	0.04	0.5
VI	III	1,500	22	42	0.08	0.6

selected from the chicken experiments are smaller than the differences between the groups from the turkey experiments. It must be borne in mind that in all of the examples from the chicken experiments, the differences in the numbers of eggs and larvae fed were comparatively small. Therefore, large differences in percentage development should not be expected.

The cause for such a relationship, as described in this paper, between the amount of infective material fed and the percentage development can only be surmised. A plausible explanation is that the larger dosages of eggs and larvae induced a stronger host reaction than did the smaller dosages. The newly acquired resistance could be directed against the organisms that induced it with the consequent elimination of some or all of them. A smaller initial dosage could induce a milder host response and allow more of the worms to survive. There is no evidence that crowding should be considered as a factor influencing the results.

A similar relationship between the number of eggs or larvae fed and the percentage development has been noted by other workers. Ackert, Graham, Nolf and Porter (1931), working with *Ascaridia galli*, fed initial doses of 500, 100, 50 and 25 eggs to chickens of the same age. Their results indicated clearly that the smaller the

number of eggs fed the larger was the percentage survival. They stated that the cause of this relationship was problematical, but suggested that it might have a serological basis. Winfield (1933), working with *Heterakis spumosa* in rats, fed graded doses of from 50 to 5,000 eggs and found that there was a consistent inverse relationship between the number of eggs fed and the percentage development. He indicated that the protective reaction of the host apparently caused the reduction in the number of worms. Sheldon (1937), working with *Strongyloides ratti*, observed that "the percentage development progressively decreased with increasingly larger doses up to 12,000 larvae. A dose of 14,000 larvae was followed by death and a dose of 15,500 larvae by a very high percentage of development. Doses of from 350 to 8,000 larvae yielded gradient numbers of worms which when plotted rise in an almost straight line. Doses of 10,000 and 12,000 larvae, instead of increasing the worm burden, actually produced markedly fewer worms. Doses of 14,000 and 15,500 appeared to cause a breakdown in the host-protective mechanism. . . ." He stated that "the marked reduction in the number of worms recovered following infection with 10,000 and 12,000 larvae is perhaps explainable on the basis of a rapidly developing acquired resistance."

The results of the experiments reported in this paper indicate that the gapeworms of the initial infection were lost rapidly, so that between the sixth and eighth weeks after being introduced only a relatively small proportion of the worms which had survived the first weeks remained. In experiment I there was an average of 22.6 pairs of worms per bird 37 days after infection, whereas 19 days later there was only an average of 2.3 pairs per bird. In experiment III, the pheasants lost almost all of their worms within 41 days after infection, although an examination of a number of these birds a few days after the worms had reached the trachea revealed that most of the birds carried heavy infections. The results of the experiments with chickens show that they lost their worms rapidly between the 14th or 15th day and the 28th day after infection. This loss in worms may be partly attributed to the development of age resistance, but acquired resistance must also have been a contributing factor. Investigations by Wehr (1939) have shown that gapeworms may survive in the turkey for 224 days and in chickens for 147 days. This would indicate that the loss of worms within a few weeks after infection cannot be attributed to the shortness of the worm's life span. It is rather a reflection of the development of an effective resistance by the host.

SUMMARY

Evidence is presented to show that turkeys, ring-necked pheasants and chickens developed marked resistance to infection with the gapeworm, *Syngamus trachea*, as a result of single infections. The resistance in turkeys was tested while worms of the immunizing infection were still in the trachea. Results of the pheasant and chicken experiments suggest that in these host species the resistance may persist after the immunizing infection has been lost.

Turkeys and pheasants of the same age were about equally susceptible to infection. However, the pheasants lost their infection more quickly than did the turkeys. On the other hand, even when very young, chickens were more resistant than either of the other host species.

In every case where comparison could be made the percentage development varied inversely with the number of eggs and larvae administered. This is assumed to

indicate that the larger doses induced a more rapid or a stronger response on the part of the host with the consequent elimination of some or all of the worms. Smaller doses induced a milder reaction and consequently a greater proportion of the worms were able to survive.

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THE GENUS *AMBLYOMMA* (IXODIDAE) IN THE UNITED STATES*

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INTRODUCTION

The publication of a review of the genus *Amblyomma* in the United States has become desirable because of the increasing interest in the members of this genus as known or suspected carriers of diseases and especially because of the need for criteria for the specific differentiation of the larvae and nymphs.

This genus, which is far richer in species than any other, is geographically limited to that portion of the world which is between the 40th parallels of latitude, that is, to the warmer countries. In the United States it is essentially limited to the South-eastern and Southcentral States.

C. L. Koch (1844) was the first to publish a comprehensive account of the ticks. He described many new species and the genus *Amblyomma*. Though he laid the foundation for the systematic classification of ticks, he failed in many instances to select adequate characters. G. Neumann in 1896 began his *Révision de la Famille des Ixodes* which included *Amblyomma* and he was the first to use satisfactory morphological characters and thereby made a definite contribution to the classification of genera and species.

Dönitz (1909) urged the importance of the ornamentation as an aid in recognizing the species and his plan of giving names to the constituent spots and stripes brought order into the terminology of the ornate pattern which most species of *Amblyomma* display. However, the species of *Amblyomma* in the United States are readily separated by morphological characters, hence the ornate color patterns are not described in this paper. They are shown in the figures.

L. E. Robinson (1926) gives excellent descriptions and figures of the 86 species then known and also summarizes their geographical distribution, hosts, economic aspects, and relation to disease.

The more important later writings include Sharif (1928), Dunn (1934), Aragão (1936) and Osorno-Mesa (1941). Other contributions have been made by Warburton (1927), Schulze (1932, 1933, 1936), Kishida (1935) and Cooley and Kohls (1942).

The collection of the Rocky Mountain Laboratory now contains 65 described species of *Amblyomma* from various parts of the world. A study of these and of other available species has led to a fuller understanding of critical morphological and color characters as well as of variation. Variation occurs, particularly in the color patterns, but is less confusing than in some other genera.

A single male specimen of *Amblyomma ovale* (Koch, 1844) taken from a dog, Sept. 8, 1941, Tama Indian Reservation, west of Tama, Iowa, was sent to us for

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identification. *A. ovale* is known from Mexico and South America. The finding of this one specimen in Iowa naturally raised the question of whether this species is indigenous in that State. Concerning this question, Mr. Eddy, the sender, has written us the following:

Relative to the establishment of *A. ovale* in Iowa, I might say that we have no further records. A special attempt was made to collect in the area where the tick was picked up and also in other near by areas. Considering the number of animals that were examined throughout the spring, summer and fall of 1941 and the fact that such animals were taken from this same area, I personally feel that the tick is not established on the Tama Iowa Indian Reservation. I realize that certain animals not checked could serve as normal hosts for this tick in North America, but I feel justified in believing otherwise.

The present paper is based largely upon miscellaneous collections made by members of the staff of the Rocky Mountain Laboratory. The authors wish to express their appreciation to these staff associates and to numerous other professional workers and various institutions in this country that have sent us materials; especially Dr. E. A. Chapin, of the U. S. National Museum; Dr. J. C. Bequaert, of Harvard University; Dr. F. C. Bishopp, of the Bureau of Entomology and Plant Quarantine; to Dr. Paul L. Piercy, of the Agricultural and Mechanical College of Texas; and to Dr. R. R. Parker, Director of the Rocky Mountain Laboratory, for advice and for reviewing this manuscript.

THE MEDICAL AND VETERINARY IMPORTANCE OF *Amblyomma* IN THE UNITED STATES

Maver (1911) reported the experimental transmission of the Rocky Mountain spotted fever rickettsia by *Amblyomma americanum* nymphs and adults which as larvae had engorged on an infected guinea pig.

Parker, Philip, and Jellison (1933) demonstrated the survival of this rickettsia from infected female ticks to the larvae of the next generation and transmission by the latter. They considered that the experimental data and the habits and host relationships of *A. americanum* strongly suggested that it was a natural vector of spotted fever. Several instances of possible transmission by this tick in Virginia, Missouri and Louisiana were cited.

In an unpublished paper presented before the American Epidemiological Society on March 20, 1942, Hassler, Sizemore and Robinson discussed seven cases of spotted fever which occurred in 1941 within a period of 32 days in an Oklahoma family in which the circumstantial evidence pointed to *A. americanum* as the vector.

Anigstein (1942) reported four Texas cases in which circumstantial evidence again pointed to *A. americanum* as the vector.

Parker, Kohls, and Steinhaus (1943) reported the recovery of the spotted fever rickettsia from nymphal *A. americanum* collected in Oklahoma. This demonstration of spontaneously infected ticks in nature, together with the accumulated circumstantial case evidence, has definitely established this tick as the third proved vector of spotted fever to man in this country.

A. americanum is certainly a potential transmitting agent of tularemia and very possibly is an actual one. However, naturally infected ticks have not been found. In unreported experiments of the Rocky Mountain Laboratory the survival of *Pasteurella tularensis* from the larval to the adult tick was shown (Parker, Philip, Davis and Cooley, 1937).

While no case of American Q fever definitely diagnosed as such has been reported following tick bite, the infectious agent, *Rickettsia diaporica*, has been recovered from several species of ticks. Parker and Kohls (1943) have recorded the recovery of ten strains from *A. americanum* collected in Liberty County, Texas, in 1937.

An apparently new clinical entity designated as Bullis fever has recently been described by Woodland, McDowell and Richards (1943) as occurring in 1942 and 1943 among troops stationed at Camp Bullis, Texas. Epidemiological data suggest that the disease is transmitted by a blood-sucking parasite and that the most likely vector is *A. americanum*.

Parker, Kohls, Cox and Davis (1939) have reported the recovery of a rickettsia pathogenic for guinea pigs from *Amblyomma maculatum* collected in Liberty County, Texas. This rickettsia belongs to the Rocky Mountain spotted fever group and is most closely related to that of boutonneuse fever. This rickettsia is apparently wide spread in the *A. maculatum* population in the South and since its initial isolation has been recovered several times from ticks collected in Texas and also from ticks collected in Georgia. The disease which it produces in guinea pigs is relatively mild. Its pathogenicity for man is unknown. If human infection is caused, it is likely of infrequent occurrence since this tick only occasionally attaches to man.

Woodland, McDowell and Richards (1943) suggested that *Amblyomma americanum* is the vector of an apparently new disease entity designated as Bullis fever, which was prevalent in 1942 and 1943 among troops stationed at Camp Bullis, Texas. Anigstein and Bader (1943a, b) have reported the recovery of a possible rickettsia, which they believe to be associated with this disease from *A. americanum* collected at this same camp.

While *A. cajennense* is a carrier of Brazilian spotted fever of Brazil and of Tobia petechial fever of Colombia, disease conditions which are presumably identical to each other and to Rocky Mountain spotted fever, it has never been found naturally infected with the spotted fever rickettsia in the United States, nor has it been suspected of being a vector. However, it is certainly a potential one. Fortunately, its distribution in this country, as at present known, is limited to the southern tip of Texas, and it appears unlikely to extend its range consequentially.

Because of their very long hypostomes, *A. cajennense* and *A. americanum*, the larvae, nymphs and adults of which all attack man freely, are particularly annoying pests in sections of the country in which they are abundant. When they are removed by pulling, the hypostome is usually left in the skin and is not pulled out as ordinarily happens with our species of *Dermacentor*. The bites of *A. americanum* have been found particularly annoying during the past two years by troops training at some of the Army camps in the South Central States, and in those where this tick has been particularly abundant morale has been noticeably affected.

The following quotation from a letter received from Mr. E. B. Palmer, of Seattle, offers first-hand evidence of the degree of annoyance that these ticks can cause. In this instance the species concerned is almost certainly *A. cajennense*.

I am going to Mexico on November 1st and expect to be there some ninety days hunting jaguar and ocelot. This will be my third trip. The first trip was down near Tampico, the next near Mazatlan and this trip will be near San Ignacio. On both previous trips I suffered the tortures of the damned with the so-called "ticks." Was unable to get its name but the Mexicans call them ticks. . . . On both my trips I was completely covered with them, particularly on my arms and legs. This little fellow is so small that you do not feel him crawl. Where he bites and

after he fills himself with blood the place where he sucked the blood forms a watery blister about the size of a pin-head, and itches so that you have to scratch it. . . . My legs and arms were raw from these bites and my scratching them. I have found that alcohol rubbed on them relieves the itching very materially. The Mexicans who accompanied me on these trips would take dry palm leaves and set them afire and singe themselves much the same as we do chickens or turkeys. They would do this once a day or more. . . . It would be a Godsend to all people hunting in Mexico if they could be relieved from the itching of these ticks.

In the United States the *Amblyomma* are not known to be important as transmitting agents of disease among domestic animals. It is likely that *A. americanum* transmits spotted fever and possibly tularemia to dogs and sheep, but in general such transmission would be of most consequence as a factor in the natural maintenance of the disease agents concerned. However, serious illnesses as a result of tularemia infections are quite possible. These ticks are most important in relation to domestic animals because of debilitation caused by massive infestations. *A. maculatum* is particularly important because "it creates conditions in ears of domestic animals which induce screwworm infestations." (Hixson, 1940.) It is likely that *A. americanum* also functions in the same manner to some extent. Hooker, Bishopp and Wood (1912) wrote:

In the sections where this tick (*A. maculatum*) occurs in any numbers it is the source of great annoyance to domestic animals, particularly to cattle. By attaching to the inside of the ears, as frequently occurs, great irritation is caused; . . . The injury in the ears furnishes opportunity for the screw-worm fly (*Chrysomya macellaria*) to deposit its eggs, which in the case of equines sometimes results in the destruction of the cartilage, thus causing the ears to droop—a condition known as "gotched" ears. The species is also of some importance on account of the fact that it sometimes attacks man.

Robinson (1926), writing of *Amblyomma* in general, stated:

Leaving out of account the question of disease transmission in the strict sense, many of the species of the genus *Amblyomma* cause severe injury to domestic stock—bovines, equines, sheep and goats—by the enormous quantities of blood which they abstract from their hosts when present in large numbers, and by the suppuration of the wounds which they inflict, and by general tick worry.

The spontaneous bacterial flora of *A. americanum* has been found by Dr. Edward A. Steinhaus, of the Rocky Mountain Laboratory, to consist of species of the genera *Bacillus*, *Micrococcus*, *Proteus*, *Alcaligenes* and *Eberthella* (unpublished data).

TERMS

Apical ventral spur.—The ventral spur at the distal end of the tarsi of adults and rarely in the nymphs of *Amblyomma*. It may be present or absent. Other more proximal ventral spurs may be present.

Basis capituli.—(Sometimes abbreviated to "basis.") The basal portion of the capitulum, spoken of as the "basal ring" by some authors. The basis capituli is movably attached to the anterior part of the scutum and lies partly within the emargination.

Capitulum.—The anterior portion of a tick which bears the mouth parts, sometimes spoken of as the "false head," "head" or "rostrum." It consists of the basis capituli, the palpi, hypostome, and chelicerae.

Cornua.—In *Amblyomma* this useful term is confusing because of the shape and contour of the basis capituli. Cornu (pl. cornua) as herein used means the more or less projecting postero-lateral corner of the basis.

Coxal spurs.—Retrograde processes on the coxae. Internal spurs are on the side toward the median line of the tick and external spurs are on the outer side toward the margin of the body.

Festoons.—Uniform, more or less rectangular areas along the posterior border of the dorsum, separated by grooves. Present in both sexes.

Files.—The longitudinal rows of the denticles or “teeth”—in this paper sometimes referred to as lateral files and median files. The lateral files indicate the outer paired rows. The median files include all except the laterals. Files number 1 are the same as the laterals, the highest numbered files are those nearest the middle line.

Frame.—The elevated chitinous periphery of the spiracular plate. When the “thickness” of the frame is described, reference is made to the transverse thickness.

Hypostome.—The ventral mouth part with recurved teeth or denticles arranged in longitudinal rows or files. The length of the hypostome is the measurement from the base to the tip and “about” is used because there is usually no definite point or line at the base from which to measure. The denticles are expressed in a formula as $3/3$ or $4/4$, indicating the number of files on each half of the hypostome. The denticles may be long and sharp or short and rounded apically. The term “over hang” is sometimes used to indicate the extension beyond the base of the tooth where it is attached.

Lateral groove.—In males, the groove at the sides of the scutum which may be complete, incomplete, or absent.

Legs.—Measurements of the length of articles are made from the dorsal side rather than from the ventral side which “telescopes.”

Macula.—In adult ticks, the more heavily sclerotized spot on the spiracular plate through which the principal respiratory opening passes.

Marginal groove.—In females the groove at the sides of the postscutal area of the body; present in unfed ticks and disappearing as the female becomes distended with feeding.

Millimeters.—All measurements are in millimeters expressed in numerals without repeating the usual “mm.”

Nubs.—Small projections on the posterior edge of the ventral scute.

Ornate.—Adults and nymphs of *Amblyomma* are either ornate or inornate, which refers to the presence or absence of a pattern of colors which include dark and light units. The pattern may be in connected lines and broad areas or in separated spots or with both. Emphasis is on the pattern rather than on the color, which varies somewhat. While an extended terminology of the members of the pattern was erected by Dönitz (1909) and revised by Robinson (1926), such terminology is omitted in this paper because it is not needed in distinguishing between the United States species. The reader is referred to the particular figures in which the pattern is shown.

Salience.—A definite edge which projects more or less.

Trochantal spur.—Spur on the ventral surface of the trochanter, similar to coxal spurs.

Ventral plaques.—More or less definite sclerotized plates situated just anterior to the ventral scutes. Present in most males of *Amblyomma* but of little use in differentiating United States species.

Ventral scutes.—Chitinous thickenings of the ventral surface of the festoons which may be very distinct and protruding, faint or absent. They may also bear nubs.

GENUS *Amblyomma* C. L. KOCH 1844

1844. *Amblyomma* Koch, p. 223.
 1872. *Adenopleura* Macalister, p. 287.
 1877. *Xiphiastor* Murray, p. 201.
 1899. *Amblyomma* Koch: Neumann, p. 200.
 1908. *Amblyomma* Koch: Banks, p. 37.
 1911. *Amblyomma* Koch: Neumann, p. 53.
 1926. *Amblyomma* Koch: Robinson, p. 9.

Usually ornate with dark spots and stripes on a pale background. Eyes and festoons present. Palpus usually long with article 2 especially long. Basis capituli of variable form, often sub-quadrangular or sub-triangular. Males without the adanal shields found in *Rhipicephalus*, *Boophilus* and *Hyalomma* though they may have ventral plaques or ventral scutes which may be extended beyond the margin of the body. Spiracular plates sub-triangular or comma-shaped. Nymphs resemble adults but may differ in the shape of the spiracular plate and are seldom ornate.

Genotype: Amblyomma cajennense (Fabricius, 1787.)

Key to Females

		PAGE
1. Scutum, inornate	<i>inornatum</i>	105
Scutum, ornate	2	
2. Coxa I with external spur distinctly longer than the internal spur	3	
Coxa I with subequal spurs	5	
3. Scutum with the pale markings usually limited to a spot near the posterior end	<i>americanum</i>	87
Scutum with pale markings in an extensive pattern	4	
4. Coxa I with internal spur about half the length of the external spur	<i>cajennense</i>	83
Coxa I with the internal spur very short or insignificant ..	<i>maculatum</i>	94
5. Coxa IV with the external spur longer than the internal spur	<i>dissimile</i>	99
Coxa IV with the two spurs about equal	<i>tuberculatum</i>	102

Key to Males

		PAGE
1. Scutum, inornate	<i>inornatum</i>	106
Scutum, ornate	2	
2. Coxa I with the internal spur moderately long	3	
Coxa I with internal spur short or insignificant	4	
3. Scutum with the pale markings in an extensive, connected pattern	<i>cajennense</i>	84
Scutum with the few pale markings in isolated spots	<i>americanum</i>	88
4. Coxae II, III, and IV each with one spur	<i>maculatum</i>	95
Coxae II, III, and IV each with two spurs	5	
5. Coxa IV with the external spur distinctly longer than the internal spur	<i>dissimile</i>	100
Coxa IV with both spurs short	<i>tuberculatum</i>	102

Key to Nymphs

		PAGE
1. Scutum, ornate	<i>tuberculatum</i>	103
Scutum, inornate	2	
2. Basis capituli pointed at the sides	<i>maculatum</i>	95
Basis not pointed at the sides	3	
3. Hypostome with dentition 3/3	<i>dissimile</i>	102
Hypostome with dentition 2/2	4	
4. Scutum with conspicuous punctations and with surface pebbled	<i>avicolens</i>	107
Scutum with moderate punctuation or with none and with surface smooth	5	
5. Scutum with punctations distinct, deep, grooves shorter and a little wider	<i>americanum</i>	88
Scutum with punctuation faint, shallow, grooves longer, and a little narrower	<i>cajennense</i>	84

The nymphs are easily separated except those of *americanum* and *cajennense*. Nymphs of *inornatum* have not been seen.

Amblyomma cajennense (Fabricius, 1787)

(Figs. 1 and 2)

1787. *Acarus cajennensis* Fabricius, p. 372.
 1794. *Ixodes cajennensis* (Fabricius), p. 427.
 1805. *Ixodes cajennensis* (Fabricius), p. 354.
 1821. *Ixodes crenatus* Say, II, p. 76.
 1844. *Amblyomma cajennense* (Fabricius) : Koch, p. 226.
 1844. *Amblyomma tenellum* Koch, p. 227.
 1844. *Amblyomma mixtum* Koch, p. 227.
 1887 (1884?). *Ixodes herrerae* Duges, p. 487.
 1888. *Amblyomma sculptum* Berlese, p. 192.
 1899. *Amblyomma parviscutatum* Neumann, p. 208.
 1899. *Amblyomma cajennense* (Fabricius) : Neumann, p. 205.
 1907. *Amblyomma cajennense* (Fabricius) : Hunter and Hooker, p. 60.
 1908. *Amblyomma versicolor* Nuttall and Warburton, p. 407.
 1912. *Amblyomma cajennense* (Fabricius) : Hooker, Bishopp and Wood, p. 151.
 1926. *Amblyomma cajennense* (Fabricius) : Robinson, p. 288.

Female

Body: Length of unengorged specimens from 3.12 to 3.42, width from 2.34 to 2.55. Oval, with the width over length varying considerably. Scutum extending about half the length. Marginal groove complete and continuing across the basis of the festoons. Nubs on the ventral scutes sometimes visible from above. Well engorged females may become as large as 13.50 × 11.00 and are about equally broad on both ends.

Capitulum: Length from 1.11 to 1.5; width of basis, 0.66. Basis sub-rectangular and with very mild corhua. Porose areas small, nearly circular. Surface smooth, shining, punctate. Palpi long, compressed laterally. Hairs moderate in number.

Scutum: Length 1.62 to 1.86, width 1.71 to 1.86. Triangular, usually a little broader than long, sometimes with length and breadth equal, widest in front of the middle, postero-lateral margins nearly straight. Cervical grooves short, deep in front, then continuing posteriorly as shallow valleys. Scapulae narrow. Eyes moderate in size, pale and very mildly convex. Ornate with whitish pattern variable. Punctations moderate in number, usually larger in the antero-lateral areas.

Legs: Moderate in length and size, relatively slightly longer and smaller than in the male. Distal end of tarsus I with apical and sub-apical ventral spurs absent, present on II, III and IV. Length of tarsus I, 1.05; metatarsus, 0.75. Length of tarsus IV, 0.66; metatarsus, 0.75.

Coxae: Coxa I with two distinct spurs, the external spur much longer. Coxae II, III and IV each with a single, broad, flat, rounded spur. All coxae with a few, long, fine hairs.

Spiracular plate: Frame moderately well sclerotized, surface a little concave at the macula. Length 0.51, width 0.51.

Genital aperture: Situated at about the level of the intervals between coxae II and III.

Hypostome: Spatulate, long, notched apically. Dentition 3/3. Length 0.93.

Male

Body: Length from 2.34 to 3.12, width 1.80 to 2.40. Robinson (1926) gives the length as 3.9 to 4.9 and width 2.3 to 3.0. Broad oval, wider behind.

Scutum: Smooth, shining, and with the dark-colored spots slightly elevated over the light-colored ones; scapulae pointed. Cervical grooves short and deep. Lateral grooves distinct posteriorly and across the festoons, fading out back of the eyes. Conspicuous extensions of the ventral plaques present in some specimens. Eyes small, and slightly elevated. Ornamented, with a characteristic pattern which includes spots on some of the festoons. Punctations moderate in size and number, the larger ones limited mainly to the light-colored areas.

Capitulum: Length from 0.84 to 1.05, width of basis from 0.48 to 0.66. Basis subquadrate, sides a little convex, cornua short to moderate. Surface smooth, shining, punctate. Palpi long, compressed laterally. Combined length of I and II, 0.51 to 0.54.

Hypostome: Spatulate, long, apically rounded and very faintly notched. Dentition 3/3. Length about 0.72.

Legs: Moderate in length, with their sizes progressively increasing from I to IV. Terminal and sub-terminal ventral spurs absent on tarsus I; present on II, III and IV. Length of tarsus I, 0.87; metatarsus, 0.66. Length of tarsus IV, 0.60; metatarsus, 0.78.

Coxae: Coxa I with two long spurs, the external one longer. Coxae II and III each with one short, broad, flat spur. Coxa IV with one long, internal spur.

Spiracular plate: Large, moderately well sclerotized, with the surface slightly concave. Length 0.66, width 0.36.

Genital aperture: Situated at about the level of the intervals between coxae II and III.

Nymph

Body: Broad oval. Length (unengorged) 1.38, width 1.14.

Capitulum: Length 0.42, width of basis 0.3. Basis sub-quadrate, sides convex, cornua faint or absent, posterior margin concave or nearly straight. Surface smooth, impunctate. Palpi long and with a few fine hairs. Combined length of articles 2 and 3, 0.24.

Hypostome: Spatulate, moderately long, faintly notched apically. Dentition 2/2. Length about 0.24.

Scutum: Length 0.66; width 0.84. Broadly cordiform, wider than long, broadest at about the middle, scapulae rounded. Cervical grooves deep and long, reaching to near the postero-lateral margins. Eyes large, pale, mildly convex. Punctations moderate in number, small, faint, seen best in reflected light.

Legs: Ventral spurs absent on distal ends of all tarsi. Length of tarsus I, 0.42; metatarsus, 0.24. Length of tarsus IV, 0.30; metatarsus, 0.21.

Coxae: Coxa I with two distinct spurs, the internal one shorter. Coxae II, III and IV each with a single spur. All spurs short, those on II, III and IV flat and relatively a little narrower than in *americanum*.

Spiracular plate: Large, nearly flat, but sunken at the respiratory opening. Length 0.21, width 0.14.

Larva

Scutum: Length 0.235, width 0.336. Much wider than long. Cervical grooves shallow, long, and about parallel. Surface smooth, faintly shagreened, impunctate.

Capitulum: Length 0.18, width 0.15. Basis short and broad, with rounded points at the sides. Surface smooth, shining, impunctate. Palpi long, with articles 2 and 3 about equal, hairs few and fine. Combined length of articles 2 and 3 about 0.11.

Hypostome: Short and broad distally. Dentition 2/2. Length about 0.086.

DISTRIBUTION AND HOSTS

The type locality of *A. cajennense* is Guiana. Robinson (1926, p. 51) summarized the known distribution of *A. cajennense* and included the previous data of Koch (1844), Stoll (1888-1893), Berlese (1888), Neumann (1899), Banks (1908),

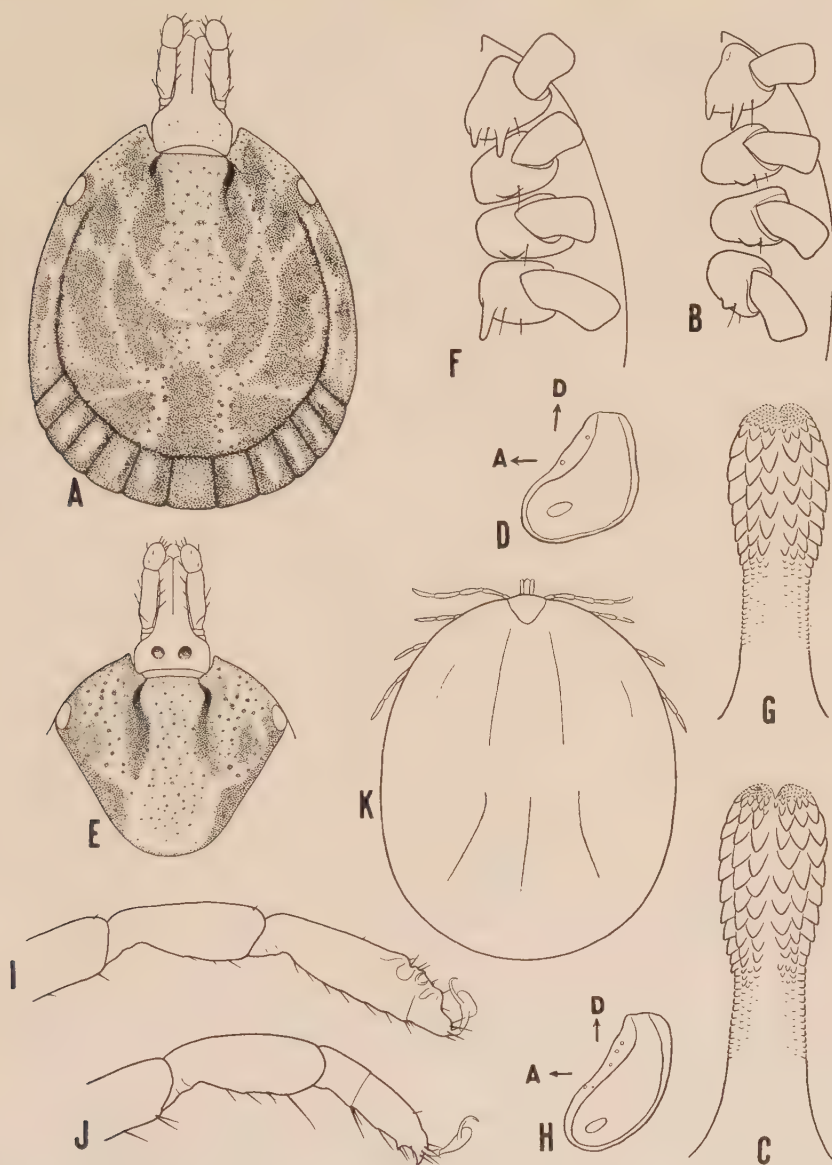


FIG. 1. *Amblyomma cajennense* (Fabricius). A. Capitulum and scutum, male. B. Coxae of female. C. Hypostome, female. D. Spiracular plate, female. E. Capitulum and scutum, female. F. Coxae of male. G. Hypostome, male. H. Spiracular plate, male. I. Leg I, female. J. Leg IV, female. K. Engorged female.

Hunter et al (1907) and Aragão (1911). He reported the species as occurring in Southern United States, Central America, West Indies and South America, particularly along the Atlantic seaboard as far south as Buenos Aires. The following countries other than the United States are definitely mentioned: Mexico, Brazil, Guatemala, Nicaragua, Costa Rica, Colombia, Guiana, Panama, Honduras, Cuba, Trinidad, Vera Cruz, Yucatan, Venezuela, Paraguay and Argentine Republic. Hunter et al (1907) include Bermuda. Banks (1908, p. 42) and Hooker et al (1912, p. 152) indicate this tick to be established in the United States only in the

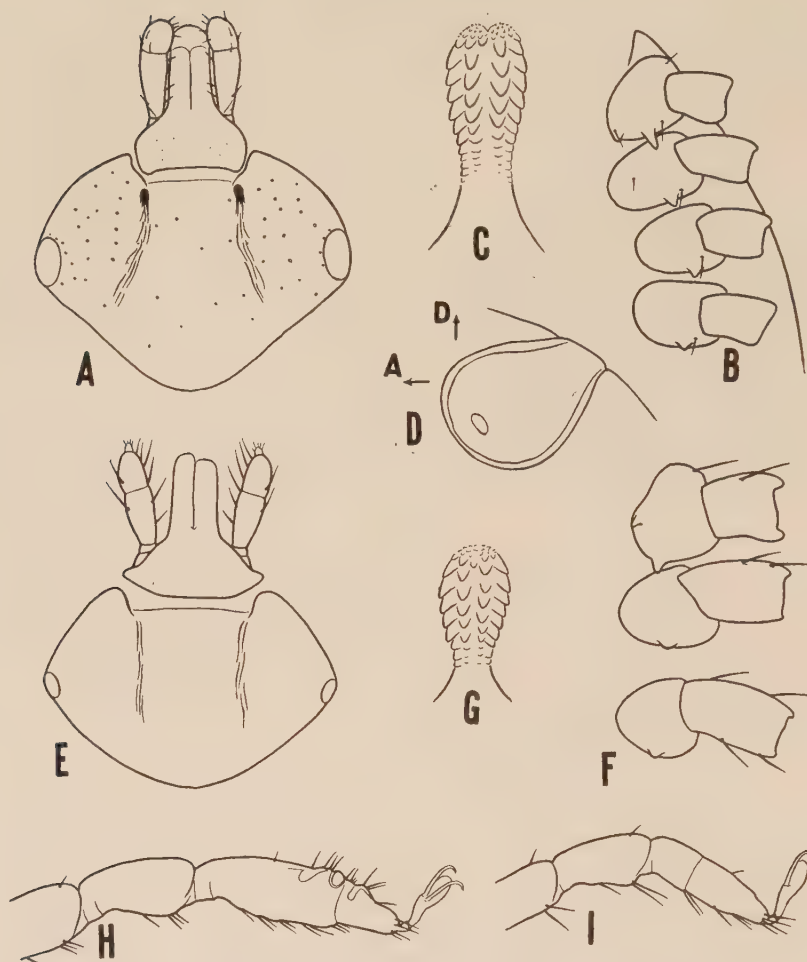


FIG. 2. *Amblyomma cajennense* (Fabricius). A. Capitulum and scutum, nymph. B. Coxae of nymph. C. Hypostome, nymph. D. Spiracular plate, nymph. E. Capitulum and scutum, larva. F. Coxae of larva. G. Hypostome, larva. H. Leg I of nymph. I. Leg IV of nymph.

southern end of Texas and southern point of Florida. References by Banks (1908), and others, and unpublished records of the Rocky Mountain Laboratory show that specimens of this tick have been found in scattered localities in several States. The Laboratory also has specimens from several of the countries listed above, as follows: From Mexico on man, opossum, *Sciurus*, and domestic animals, 17 lots; from Colombia on domestic animals, 8 lots; from Brazil on man, domestic animals, and capybara, 16 lots; from Honduras on domestic animals, 5 lots; from Nicaragua on man and domestic animals, 6 lots; from the Canal Zone, hosts not stated, 4 lots. Including the records of previous writers, some 25 different animals have been reported as hosts.

Dr. Paul L. Piercy has kindly shown us the extensive records of his survey of ticks in Texas and it seems perfectly evident that the tick is abundant and established only in the following counties in Texas: Nueces, Jim Wells, Kleberg, Brooks, Kennedy, Starr, Hidalgo, Willacy and Cameron. From the Banks' record it is apparent that *A. cajennense* was at least formerly present in the southern point of

Florida. However, there have been no records published subsequently of the presence of this species in Florida. In a recent letter from Homer Hixson, he states:

The status of the tick *Amblyomma cajennense* in Florida is apparently not definitely known. Although I have not known of any intensive collecting in Southern Florida, the collecting I have known about indicates that this tick does not occur in Florida or if it does, it is rare.

A. cajennense commonly attacks man, horses, cattle, sheep, goats, dogs and pigs. Neumann (1909, p. 442) mentions several authentic records of its occurrence on the tongues of young calves and gives one record on *Bufo marinus*.

Records of the Rocky Mountain Laboratory for the United States are given in the accompanying table.

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
10021	Tex.	Brownsville, Cameron Co.	2/8/34	Goat	318 A.	L. Demieville
10048	"	"	4/14/34	?	372 A.	"
10036	"	"	Mar. 1935		23 N., 76 A.	"
11062	"	"	Apr. 1935	Goat	90 A.	"
11060	"	"	4/13/35	Cow	65 A.	"
11061	"	"	"	Horse	105 A.	"
11087	"	"	May 1935	Cow	100 A.	"
11063	"	"	5/11/35	Goat	165 A., 30 N.	"
11119	"	"	June 1935	Cow	10 A.	"
14227	"	"	5/3/38	Dogs	?	R. M. L.
14229	"	"	"	Horses	110 A.	"
14232	"	"	5/4/38	"	100 A.	"
14234	"	"	5/5/38	"	100 A.	"
14235	"	"	"	Drag	125 N., 30 A.	"
20479	"	"	9/14/42		2 A.	St. Bd. Health
11436	"	Kingsville, Kleberg Co.	10/16/35	Peccaries	3 N., 10 A.	R. M. L.
11442	"	"	10/18/35	Drag	20 A.	"
14183	"	"	5/25/38	Deer	3 A.	R. M. L.
14833	"	"	7/25/38	Peccaries	1 A.	J. C. Brown

Amblyomma americanum (Linnaeus, 1758)

(Figs. 3 and 4)

1758. *Acarus americanus* Linnaeus, p. 615. (Not *Acarus americanus*, *A. nigua*, from Brazil, of Treviranus, 1831, pp. 185-191. See Salmon and Stiles 1901, p. 475.)
1778. *Acarus nigua* de Geer, p. 153.
1804. *Ixodes nigua* (de Geer) : Latreille, p. 52.
1804. *Rhynchoprion americanum* (Linnaeus) : Hermann, p. 71.
1805. *Ixodes americanus* (Linnaeus) : Fabricius, p. 356.
1844. *Amblyomma americanum* (Linnaeus) : Koch, p. 229.
1869. *Ixodes unipunctata* Packard, p. 66.
1880. "*Argas americanum* de Geer" of Megnin, p. 134 (in part).
- 1886-1893. *Amblyomma foreli* Stoll, p. 21.
1899. *Amblyomma americanum* (Linnaeus) : Neumann, p. 209.
1901. *Amblyomma americanum* (Linnaeus) : Salmon and Stiles, p. 475.
1908. *Amblyomma americanum* (Linnaeus) : Banks, p. 40.
1912. *Amblyomma americanum* (Linnaeus) : Hooker, Bishopp and Wood, p. 142.
1926. *Amblyomma americanum* (Linnaeus) : Robinson, p. 45.
1929. *Amblyomma americanum* (Linnaeus) : Oudemans, p. 198.
1936. *Amblyomma americanum* (Linnaeus) : Oudemans, p. 455.

A relatively small tick, with a considerable variation in size and also with an unusual disparity between the smaller males and larger females.

Female

Body: Length, unengorged, 2.46 to 3.40, width 1.98 to 2.60. Broad oval, with the scutum reaching about half the length. Well engorged specimens may become as large as 11.0×9.25.

Scutum: Length 3.30, width 1.80. Sub-triangular, posterior angle broad, posterior apex

flattened, postero-lateral sides nearly straight, scapulae pointed. Posterior angle with a conspicuous whitish spot with red and green tinges; sometimes with other spots in the scapular fields. Punctations numerous, rather evenly distributed and larger in the anterior areas. Cervical grooves short and deep, convergent posteriorly. Eyes flat, large, and pale.

Capitulum: Length 0.90 to 1.14, width of basis 0.60 to 0.73. Basis sub-rectangular, dorsal surface smooth, punctate, convex, with sides more declivitous; posterior margin nearly straight, salient; postero-lateral corners rounded, mildly suggesting cornua. Porose areas oval, divergent anteriorly, and separated by about the length of the longer axis of one. Palpi long, with the surface irregular. Combined length of 2 and 3, 0.84.

Hypostome: Long, spatulate, faintly notched apically; denticles 3/3. Length about 0.75.

Legs: Long and small and with long, fine hairs. Apical and sub-apical ventral spurs absent on tarsus I, present on II, III and IV. Length of tarsus I, 0.96; metatarsus, 0.66. Length of tarsus IV, 0.75; metatarsus, 0.70.

Coxae: Coxa I with two spurs, the external one long and pointed, a single broad, plate-like spur each on II and III, a single, triangular, plate-like spur on IV. Long, fine hairs on all coxae.

Spiracular plate: Large and with its frame moderately sclerotized, surface concave. Length 0.60, width 0.54.

Genital aperture: Situated at the level of the intervals between coxae II and III.

Male

Body: Length 2.95, width 2.26. Oval, wider behind.

Scutum: Smooth and shining. Surface in general flattened, more convex in front of the festoons. Cervical grooves short, deep, and convergent anteriorly. Lateral grooves distinct near the festoons, disappearing back of the eyes. Festoons long, all limited anteriorly by a continuation of the marginal groove. Ornamentation limited to symmetrical, isolated whitish spots as shown in the figure. In some specimens the white spots are much smaller or absent. Punctations numerous and small. Eyes flat, pale.

Capitulum: Length 0.9, width of basis 0.59. Basis sub-quadrate, smooth, shining, and punctate; cornua short. Palpi long, with the surface irregular. Combined length of articles 2 and 3, 0.54.

Hypostome: Essentially as in the female. Denticles 3/3. Length about 0.48.

Legs: Legs long and small, and with long, fine hairs. Apical and sub-apical ventral spurs absent on tarsus I, present on II, III and IV. Length of tarsus I, 0.69; metatarsus, 0.48. Length of tarsus IV, 0.54; metatarsus, 0.54.

Coxae: Coxa I with two spurs, the external one longer and pointed. Coxae II and III each with a single broad, flat spur. Coxa IV with a long, pointed, internal spur. All coxae with long, fine hairs.

Genital aperture: Situated between coxae II.

Spiracular plate: Large, convex, and with the frame only moderately sclerotized. Length 0.51, width 0.28.

Nymph

Body: Broad oval. Length (unengorged) 1.32, width 1.23.

Capitulum: Length 0.42, width of basis 0.30. Basis broad and short. Posterior margin nearly straight, salient. Surface smooth, shining, and with a very few punctations. Palpi long, combined length of 2 and 3, 0.21.

Hypostome: Long, rounded apically. Dentition 2/2. Length 0.27.

Scutum: Length 0.6, width 0.75. Broadly cordiform, usually slightly broader than long, broadly rounded behind. Cervical grooves deep in front, becoming shallow behind and disappearing back of the eyes. Eyes large, pale, and a little convex. Surface smooth, shagreened. Punctations moderate in number, larger, and more easily seen than in *cajennense*.

Legs: Apical and subapical ventral spurs absent on all tarsi. Length of tarsus I, 0.33; metatarsus, 0.21. Length of tarsus IV, 0.28; metatarsus, 0.19.

Coxae: Coxa I with both external and internal spurs present, coxae II, III and IV only with the external spur present. All spurs short.

Spiracular plate: Large, mildly sclerotized. Length 0.26, width 0.18.

Larva

Capitulum: Length 0.20, width of basis 0.15. Basis short, broad, and rounded at the sides. Surface smooth, shining and impunctate. Palpi short, laterally compressed, and with relatively long hairs. Length about 0.11.

Hypostome: Small, spatulate in shape, rounded apically. Dentition 2/2. Length about 0.66.

Scutum: Shape much as in the nymph, but relatively shorter. Cervical grooves moderately deep, long, parallel. Surface smooth, shagreened, impunctate. Length 0.25, width 0.35.

Coxae: Coxa I with two small spurs; II and III with one spur.

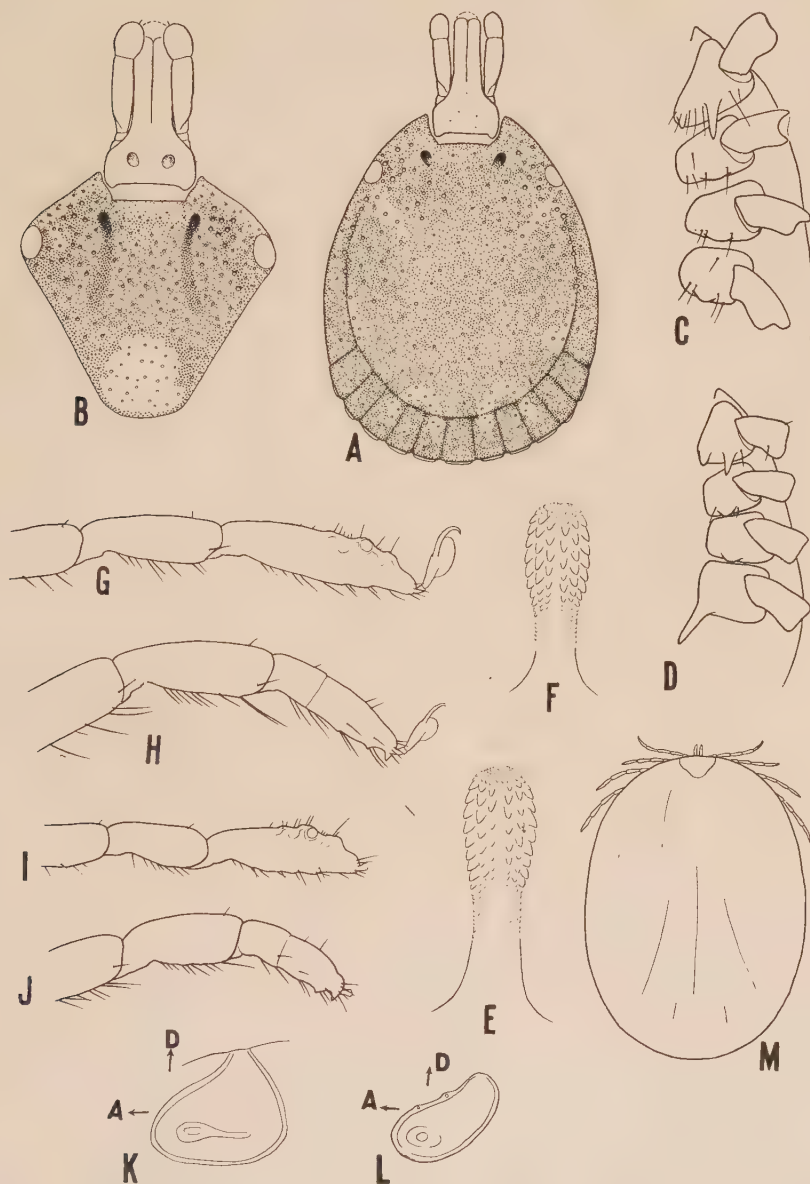


FIG. 3. *Amblyomma americanum* (Linnaeus). A. Capitulum and scutum, male. B. Capitulum and scutum, female. C. Coxae of female. D. Coxae of male. E. Hypostome, female. F. Hypostome, male. G. Leg I, female. H. Leg IV, female. I. Leg I, male. J. Leg IV, male. K. Spiracular plate, female. L. Spiracular plate, male. M. Engorged female.

DISTRIBUTION AND HOSTS

The type locality of *americanum* is Pennsylvania or New Jersey but the type host is not known.

Knowledge of the distribution of this tick in the United States is rather indefinite and we can do no better than to quote from a recent publication by Parker, Kohls and Steinhaus (1943), as follows:

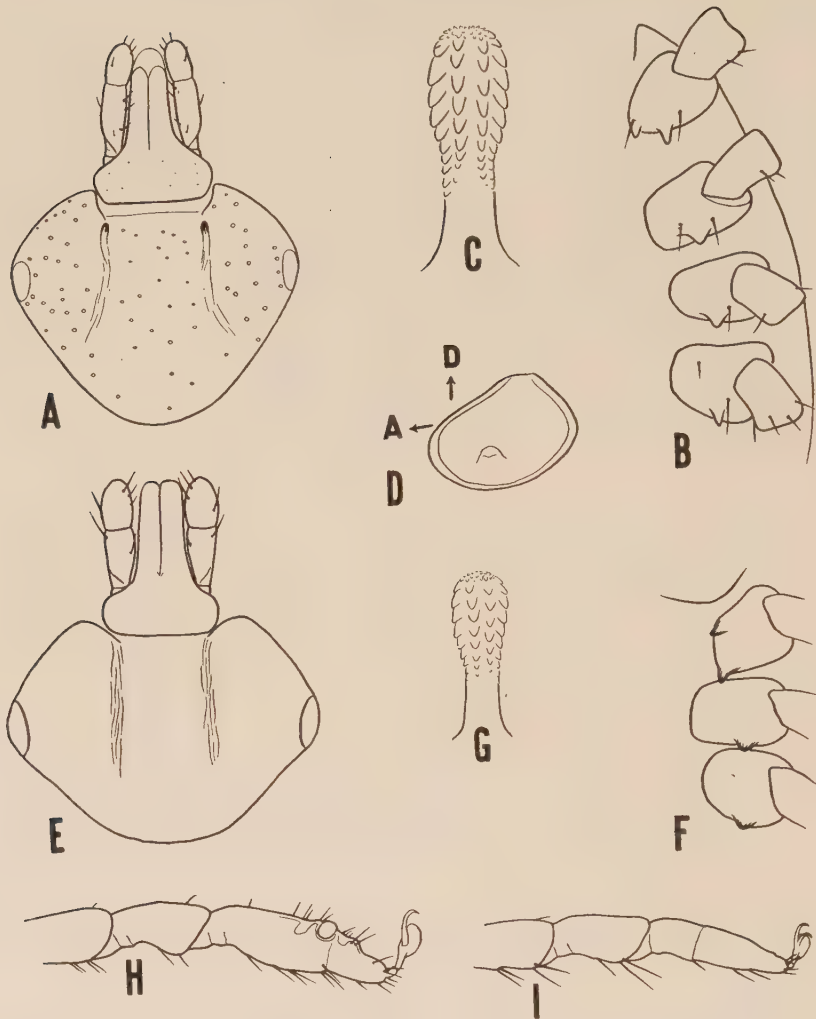


FIG. 4. *Amblyomma americanum* (Linnaeus). A. Nymph, capitulum and scutum. B. Nymph, coxae. C. Nymph, hypostome. D. Nymph, spiracular plate. E. Larva, capitulum and scutum. F. Larva, coxae. G. Larva, hypostome. H. Leg I of nymph. I. Leg IV of nymph.

Hooker, Bishopp and Wood (1912) show *A. americanum* as occurring east and south of a line starting from a short distance west of the southernmost tip of Texas and extending northward and northeastward across the States of Oklahoma, Kansas (southeastern corner), Missouri, Illinois, and Indiana into southern Michigan, and thence almost directly eastward across New York and the southern portions of the three northern New England States. However, such evidence as we have suggests that it is extremely scarce in the northern portion of this area.* We also have recent reports of its occurrence in southern Iowa. Actually, there is very little published information concerning the distribution and abundance of this tick in any of the 18 States concerned. Apparently it is most abundant in parts of Texas, Louisiana, Oklahoma, Arkansas, and Missouri. Doubtless it is abundant, at least sporadically, in other southern States eastward to the Atlantic Coast.

*The State Entomologists of Ohio, Indiana, and Illinois (J. S. Houser, J. J. Davis, and W. P. Flint, respectively), and R. E. Rebrassier, of the College of Veterinary Medicine of Ohio State University, have recently informed us that they know of no records of *A. americanum* in their respective States.

The Marx collection contained one specimen from Labrador. Neumann (1911) extended the range of the species to Guatemala, Brazil and Guiana and our records suggest it is common in Mexico.

A. americanum shows a very wide range of hosts, including birds. All three stages commonly bite man. Neumann (1911) records it on man, cow and *Felis pardalis*. Hooker et al record it on man, dog, cattle, horse, deer, goat, peccary, hog, mule, skunk, sheep, wolf, fox, squirrel, badger, domestic cat, wild turkey and chaparral cock. The records on dog, cattle, man and horse are most frequent and in that order. Our records add the cotton rat, wild hog (Mexico) and "Tinamou" (Mexico).

Rocky Mountain Laboratory records of this tick in the United States are in the accompanying table.

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
14191	Ark.	Harrison, Boone Co.	6/6/38	Dogs	104 A.	R. M. L.
14192	"	"	"	Cows	134 A., 1 N.	"
14196	"	"	6/7/38	7 dogs	2 A.	"
14813	"	Alpine, Boone Co.	6/8/38	2 horses, cow	12 A.	"
15506	"	Boone Co.	3/14/39	Dog	1 ♀	S. J. Carpenter
16653	"	Lead Hill, Boone Co.	7/5/39	Dog, cattle, horse	75 ♀, 14 ♂	"
16654	"	Boone Co.	7/7/39	Dog	82 ♀, 26 ♂	"
15520	"	Calhoun Co.	4/4/39	Cow	8 ♀	"
16655	"	Denver, Carroll Co.	7/6/39	Dog	2 N., 7 ♂, 30 ♀	"
15517	"	Sparkman, Dallas Co.	4/3/39	"	14 ♂, 14 ♀	"
15516	"	Sparkman, Dallas Co.	4/3/39	Cow	4 ♀, 2 ♂	"
19047	"	Hot Springs, Garland Co.			36 N., 22 L.	Bur. An. Ind.
20293	"	Ouachita Co.	July '43		1 N., 2 A.	W. J. Baerg
20292	"	Union Co.	"		1 A.	"
20274	"	Washington Co.	6/20/43		1 A.	"
20291	"	"	7/10-14 1943	Man	1 A.	"
19646	Ga.	Broxton, Coffee Co.	6/4/42	Dog	2 N., 3 A.	Dr. Geo. Brigham
20233	"	Blackbeard Isl. (off coast of Georgia)	7/13/42	Soil	1 A.	Herbert H. Ross
18885	Ia.	Osceola, Clarke Co.	6/28/41	Cow	1 ♀	G. S. Cantonwine
19772	La.	Kisatchie Nat. For., Grant Parish	June 1941		2 ♀	Jones & Archer
7661	Miss.	Phoenix, Yazoo Co.	7/22/31		36 ♀, 21 ♂	
13425	"	Vicksburg, Warren Co.	2/26/37	Dog	1 ♂	R. M. L.
20496	"	Harrison Co.	8/20/43	Man	1 N.	F. N. Young, Jr.
14817	Mo.	Golden, Barry Co.	Oct. 1937	Man	1 N.	H. H. Echwalt
14826	"	Cassville, Barry Co.	6/8/38	3 cottontail rabbits	47 N.	R. M. L.
14827	"	Golden, Barry Co.	6/8/38	Timber wolf	5 ♀	"
14822	"	Cassville, Barry Co.	6/9/38	12 dogs	29 A., 16 N.	"
14823	"	"	"	1 dog	14 A.	"
14828	"	"	"	12 dogs	70 N., 11 A.	"
14829	"	"	"	"	40 N., 14 A.	"
14830	"	"	"	5 dogs	47 N., 30 A.	"
14831	"	"	"	"	24 N., 40 A.	"
14824	"	Eagle Rock, Barry Co.	"	"	35 N., 36 A.	"
14825	"	"	"	"	13 N., 32 A.	"
14824	"	Golden, Barry Co.	"	Timber wolf	35 N., 56 A.	
14821	"	Viola, Barry Co.	6/10/38	8 cottontail rabbits	41 N.	R. M. L.

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
16654	"	Bergman, Boone Co.	7/7/39	Dogs	100 A.	"
20276	"	Jefferson City, Cole Co.	6/26/43	Dog	2 A.	Dr. John W. Williams, Jr., St. Bd. Health
19048	"	Hot Springs, Gariand Co.			2 N.	Dr. H. H. Little
16653	"	Lead Hill, Marion Co.	7/5/39	Dogs	8 A.	R. M. L.
14370	Okla.	Atoka, Atoka Co.	6/6/35	Dog	4 A.	"
19233	"	Durant, Bryan Co.	9/6/41	Dogs and grass	7 N.	Dr. F. R. Hassler
19929	"	Armstrong, Bryan Co.	9/4/42	Drag	180 N.	R. M. L.
19931	"	Wilson, Carter Co.	9/9/42	Cow and dog	18 N.	"
19932	"	"	"	Drag	490 N.	"
20488	"	Cache, Comanche Co.	3/4/42	Coyote	1 A.	Frank B. McMurtry
8127	"	Broken Bow, McCurtain Co.	2/20/32	Cow	1 ♀	Mr. Sanborn
20249	"	Camp Gruber, Muskogee Co.	6/1/43	Drag	3 N., 13 A.	Herbert H. Ross
20295	"	"	5/15-6/11/43	Man	14 N., 16 A.	Michael Ballin
20294	"	"	6/11 & 7/1/43	"	11 N., 8 A.	"
19933	"	Weathers, Pittsburg Co.	9/11/42	6 dogs	3 A.	R. M. L.
19934	"	"	"	Drag	110 N.	"
20287	Tenn.	Baird's Mills, Wilson Co.	7/12/43		86 A.	War Dept., Nashville
20300	"	"	8/1/43		12 N., 6 A.	"
20453	"	"	8/8/43		35 A.	"
20451	"	"	8/9/43		250 N., 1 A.	"
20457	"	"	8/10-14/43	Ground	354 N.	"
20454	"	"	8/12/43		460 N.	"
20460	"	Nashville, Davidson Co.	8/24/43		387 N.	"
20462	"	"	8/25/43		101 N.	"
20463	"	"	8/27/43		225 N.	"
20244	Tex.	Camp Hood, Bell Co.	5/30/43		1 N., 4 A.	Dr. David Lackman
20290	"	"	4/26/43	Man	3 A.	"
19881	"	West Columbia, Brazoria Co.	?		3 ♀	T. McGregor
19951	"	"	6/23/42	Man	1 N.	Dr. W. Gingrich
19952	"	"	"	"	1 N.	"
19919	"	"	8/22/42	Drag	2500 N.	R. M. L.
19920	"	"	8/24/42	Cow	1 ♀, 1 N.	"
19922	"	"	"	Drag	900 N.	"
19921	"	"	"	Dog	?	"
19923	"	"	8/25/42	Drag	2000 N., 1 ♂	"
20467	"	Brazos Co.	4/19/43	"	4 N.	State Bd. Hlth.
20472	"	Brown Co.	10/10/42	Dog	1 N.	"
17312	"	Colorado Co.	5/30/40	Deer	1 N., 4 A.	T. T. Waddel
20458	"	Dallas Co.	8/5/43		19 N.	Southwest Biological Sup. Co., State Bd. Hlth.
20464	"	Cuero, DeWitt Co.	5/21/43		5 A.	"
13922	"	Tartleton Prairie, Erath Co.	7/29/37	Cows	22 N.	R. M. L.
13924	"	"	"	"	4 A.	"
13935	"	"	8/3/37	Cow	3 A.	"
13936	"	"	"	Horse	2 N.	"
13931	"	"	8/5/37	Cows	8 N., 12 A.	"
13932	"	"	"	Dogs	20 N.	"
19924	"	Galveston, Galveston Co.	8/27/42	Dogs	1 ♀	"
20459	"	Hardin Co.			14 N.	Southwest Biological Sup. Co., State Bd. Hlth.
20465	"	Jefferson Co.	5/19/43	Dog	5 A.	J. C. Brown
14188	"	Kingsville, Kleberg Co.	5/27/38	Cottontail	1 ♂	"
14955	"	"	12/18/38	Deer	1 ♀	"
13709	"	Plum Grove, Liberty Co.	July 1937		6 A.	State Bd. Hlth.
13705	"	"	7/2/37	Grass	100 A.	"
13698	"	"	7/3/37	Cows and wooded area	5 A.	"
13692	"	"	7/4/37	Dogs	8 A.	R. M. L.
13702	"	"	7/5/37	Goats	41 A.	St. Bd. Hlth.

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
13703	"	"	7/6/37	Bushes	5 A.	"
13693	"	"	7/7/37	Goats	23 A.	R. M. L.
13695	"	"	"	Cows	11 A.	"
13696	"	"	"	Squirrel	5 A.	St. Bd. Hlth.
13701	"	"	"	Cows	7 A.	"
13489	"	"	7/9/37	Dog	5 A.	R. M. L.
13491	"	"	"	Man	4 A.	"
13492	"	"	"	Horse	28 A.	"
13490	"	"	7/10/37	Cows	41 A.	"
13716	"	"	7/12/37	Dog	5 A.	"
13715	"	"	7/14/37	Horse	9 A.	"
13719	"	"	7/14/37	Dog	6 A.	"
13712	"	"	"	Cow	39 A.	St. Bd. Hlth.
13713	"	"	"	Dogs	20 L., 4 N., 2 A.	"
13718	"	"	7/15/37	Goat	22 A.	R. M. L.
13495	"	"	7/16/37		3 A.	"
13342	"	"	7/19/37		8 A.	"
13826	"	"	7/19/37	Cow	6 A.	"
13827	"	"	"	Dog	15 A.	"
13828	"	"	7/20/37	Goats	16 A.	"
13835	"	"	7/22/37	Dog	8 A.	"
13836	"	"	"	Goat	23 N.	"
13837	"	"	7/23/37	Cow	27 N.	"
13915	Tex.	Plum Grove, Liberty Co.	7/26/37	Cows	21 N., 10 A.	R. M. L.
13916	"	"	"	Dog	19 A.	"
13919	"	"	7/28/37	"	4 A.	"
13920	"	"	"	"	21 A.	"
13921	"	"	"	Cows	27 N.	"
13923	"	"	7/30/37	"	28 A.	"
13926	"	"	"	"	10 A.	"
13929	"	"	8/4/37	"	10 N., 19 A.	"
13933	"	"			6 N., 11 A.	"
13840	"	"	8/8/37	Cow	6 N., 20 A.	"
13844	"	"	"	Drag	Many N., 1 A.	"
13841	"	Parker Place, Liberty Co.	"	"	200 A.	"
13842	"	"	"	Dog	12 N., 10 A.	"
13843	"	"	"	2 horses	20 N., 15 A.	"
13846	"	Plum Grove, Liberty Co.	8/9/37	Drag	200 N., 1 A.	"
13847	"	"	"	8 cows	2 N., 3 A.	"
13848	"	"	"	Dog	2 N.	"
13849	"	Parker Place, Liberty Co.	"	Drag	450 N., 2 A.	"
13850	"	Howard Ranch, Liberty Co.	"	12 cows	2 N., 54 A.	"
13851	"	Plum Grove, Liberty Co.	8/10/37	Goats	14 N., 2 A.	"
13852	"	"	"	Cows	95 N., 27 A.	"
13853	"	Hughes Kennels, Liberty Co.	"	Dog	27 N., 8 A.	"
13854	"	Ford Place, Liberty Co.	"	"	30 L., 2 N., 3 A.	"
13855	"	Hightower Ranch, Liberty Co.	"	"	21 L., 17 N., 3 A.	"
13857	"	Cleveland, Liberty Co.	"	2 horses	6 N., 4 A.	"
13858	"	"	"	5 dogs	100 N.	"
13859	"	Parker Place, Liberty Co.	8/11/37	Drag	100 N.	"
13861	"	"	"	"	100 N.	"
13862	"	Cleveland, Liberty Co.	"	Horse	5 N., 1 A.	"
13863	"	Parker Place, Liberty Co.	8/12/37	Drag	300 N.	"
13864	"	"	"	2 horses	15 N., 5 A.	"
13866	"	Plum Grove, Liberty Co.	"	Cows	11 N., 3 A.	"
13867	"	"	"	8 dogs	5 N., 5 A.	"
13868	"	Parker Place, Liberty Co.	"	Drag	300 N.	"
13869	"	"	"	Cow	4 N., 5 A.	"
13870	"	"	8/13/37	Drag	675 N.	"
13871	"	"	8/14/37	"	1550 N.	"
13873	"	"	"	Rabbit	Many L., 230 N., 100 A.	"
13872	"	Cleveland, Liberty Co.	"	Dogs	32 N., 7 A.	"
14247	"	"	5/11/38	6 horses	1 N., 68 A.	"
14248	"	"		12 dogs	65 A.	"

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
14252	"	"	5/12/38	15 dogs	20 A.	"
14256	"	"	"	Fox squirrel	9 N.	"
14926	"	Dayton, Liberty Co.	9/9/38	13 dogs	8 A.	"
14927	"	"	"	12 cows	1 ♀	"
19954	"	Dayton, Liberty Co.	8/15/42		19 N.	"
19953	"	Liberty, Liberty Co.	8/16/42		66 N.	"
14264	"	Cleveland, Liberty Co.	5/16/43	10 goats	73 N.	"
13494	"	Montgomery Co.	7/16/37		11 A.	St. Bd. Hlth.
20169	"	Newton Co.	4/4/43	Drag	30 A.	Melvin Kyle
13704	"	San Jacinto Co.	7/4/37	Horses	21 A.	St. Bd. Hlth.
13496	"	"	7/11/37	Cows	12 A.	"
13498	"	"	"	Horses	20 A.	"
13499	"	"	"	Dogs	6 A.	"
13714	"	"	7/14/37	Parker home	6 N., 5 A.	"
13720	"	"	7/15/37	Horse	12 A.	"
13721	"	"	"	Dog	14 A.	"
13722	"	"	"	Flag	19 N., 8 A.	"
13830	Tex.	San Jacinto Co.	7/16/37	Goat	4 A.	St. Bd. Hlth.
13831	"	"	"	Flag	4 A.	"
13832	"	"	"	Horse	15 A.	"
13833	"	"	"	Dog	12 N., 14 A.	"
13829	"	"	7/16-17-30/37	Cows	35 A.	"
13834	"	"	7/21/37	Goats	20 A.	"
13838	"	"	"		5 A.	"
13839	"	"	"	Cow	25 A.	"
13917	"	"	7/23/37	Dogs	6 A.	"
13918	"	"	7/27/37	Cows	16 N.	"
13938	"	"	8/2/37	Cow and dog	9 A.	"
13934	"	"	8/3/37	Dog	16 N., 9 A.	"
14223	"	Manor, Travis Co.	5/1/38	Mule	25 A.	"
14224	"	"	"	Horse	10 A.	"
14225	"	"	"	3 calves	21 N., 9 A.	"

Amblyomma maculatum Koch, 1844
(Figs. 5 and 6)

1844. *Amblyomma maculatum* Koch, p. 227.
 1844. *Amblyomma tigrinum* Koch, p. 227.
 1844. *Amblyomma rubripes* Koch, p. 227.
 1844. *Amblyomma ovatum* Koch, p. 228.
 1844. *Amblyomma triste* Koch, p. 229.
 1888. *Amblyomma complanatum* Berlese, p. 191.
 1908. *Amblyomma maculatum* Koch: Banks, p. 39.
 1911. *Amblyomma maculatum* Koch: Neumann, p. 70.
 1912. *Amblyomma maculatum* Koch: Hooker, Bishopp and Wood, p. 135.
 1926. *Amblyomma maculatum* Koch: Robinson, p. 40.
 1936. *Amblyomma maculatum* Koch: Oudemans, p. 504.

Female

Body: Length, unengorged, from 3.70 to 4.40, width from 2.40 to 2.85. Engorged examples may become as long as 18.00 and as wide as 13.00. Oval, a little narrower in front. Scutum less than half the body length. Marginal groove complete and continuous across all the festoons. Festoons often with a small, terminal nub visible from above.

Scutum: Length 1.8 to 2.20, width 1.60 to 2.00. Sub-triangular, narrowed posteriorly, rounded terminally; antero-lateral border very convex. Scapulae long, pointed. Eyes a little convex. Cervical grooves moderately deep anteriorly, shallow, and disappearing posteriorly. Ornate, with the light and dark color pattern contrasting sharply. Punctations numerous, large and small ones intermingled, the former more numerous in anterior areas.

Capitulum: Length from 1.00 to 1.25, width of basis 0.73. Basis sub-rectangular in shape, postero-lateral corners a little protruding, salient. Porose areas large, convex, a little divergent anteriorly. Surface smooth, shining, punctate. Palpi long, wider at article 3; hairs few and short.

Hypostome: Long, mildly notched apically. Dentition 3/3, though examination of numerous

specimens shows a tendency for a 4/4 pattern. One female examined was definitely 4/4 but the teeth of the fourth row (median) were small. Length 0.78.

Legs: Long and large, I longer but smaller than the others. Apical spurs absent on tarsus I, present on II, III and IV. Metatarsi II, III and IV with long, paired terminal spurs, directed distad. Length of tarsus I, 1.11; metatarsus, 0.99. Length of tarsus IV, 0.78; metatarsus, 1.20.

Coxae: Coxa I with a very short internal and a long, pointed external spur. Coxa II with a broad, short, flat spur; III and IV with similar spurs, but progressively smaller. All coxae with a few long, fine hairs.

Spiracular plate: Large, concave, with the frame well sclerotized. Length 0.87, width 0.66.

Genital aperture: Situated at the level of the intervals between coxae II and III.

Male

Body: Length from 3.40 to 5.00, width from 2.40 to 3.00. A medium-sized tick, oval, wider behind.

Scutum: Often with the surface mildly concave between the eyes. Lateral grooves deep, complete, starting near the eyes and with their continuation limiting the long, well-defined festoons. Ventral scutes extending over the margin and visible from above. Cervical grooves deep in front, shallow behind and disappearing back of the eyes. Scapulae long with the interval between them deep. Punctations numerous and deep in the area within the marginal groove, sparse on the festoons. Eyes a little convex. Ornate with numerous, mostly connected lineal spots of golden white (see the figure).

Capitulum: Length 0.80 to 1.20, width of basis about 0.63. Basis sub-rectangular, longer than in many species; dorsal surface convex, a little irregular, punctate; postero-lateral corners a little protruding, salient, forming short cornua. Palpi long, widest on article 3. Hairs moderate in number, short and fine. Length 0.66.

Hypostome: Spatulate, long, notched terminally. Dentition 3/3; rarely there is a fourth pair more or less complete. Length from 0.66 to 0.78.

Legs: Long and large. Leg I smaller. Apical spurs present on II, III and IV, absent on I. Long, paired, terminal spurs present on metatarsi II, III, IV, absent on I. Hairs long and fine. Length of tarsus I, 0.84; metatarsus, 0.84. Length of tarsus IV, 0.60; metatarsus, 1.02.

Coxae: Coxa I with long, pointed external spur; internal spur short, almost negligible. Coxae II and III with short, broad, flat spurs. Coxa IV with a long, pointed internal spur. All coxae with a few long, fine hairs.

Spiracular plate: Large, a little convex, with the frame well sclerotized. Length 0.69, width 0.51.

Genital aperture: Situated between coxae II.

Nymph

Body: Unengorged body pyriform, narrow; with the scutum reaching about one-third the length. Length 1.44, width 0.93.

Scutum: Length 0.57, width 0.63. Slightly wider than long, broadly rounded behind; widest back of the middle. Scapulae rounded and covered by the sides of the basis capituli. Cervical grooves moderately deep anteriorly, then continued as shallow valleys to near the postero-lateral margins. Eyes pale, nearly flat. Surface faintly shagreened. Punctations sparse. Hairs absent.

Capitulum: Length 0.36, width of basis 0.315. Basis triangular, with the lateral points protruding outside of the scapulae. Ventral surface of basis with two distinct retrograde spurs. Posterior margin convex. Surface nearly flat (dorsally), smooth, shining, impunctate. Palpus long, narrow at the base, broadest at about the suture between 2 and 3. Combined length of 2 and 3, 0.20. Hairs few and moderately long.

Hypostome: Spatulate, rounded terminally. Dentition 2/2. Length about 0.17.

Legs: Relatively a little shorter and smaller than in the adults. All tarsi with apical ventral spurs absent. Paired terminal spurs absent on all metatarsi. Length of tarsus I, 0.35; metatarsus, 0.18. Length of tarsus IV, 0.24; metatarsus, 0.21.

Coxae: Coxa I with only moderate internal spur. Coxa II with one very short, broad, flat spur. Coxae III and IV without spurs. All coxae smooth and shining and with a few fine hairs.

Spiracular plate: Large, flat, and with frame little sclerotized. Length 0.23, width 0.15.

Larva

Capitulum: Length 0.17, width of basis 0.16. Basis sub-triangular, wide, with the lateral points extending outside over the scapulae; posterior margin rounded. Surface smooth, shining, faintly shagreened, impunctate. Palpi short, smooth, and with a few fine hairs. Length 0.086.

Hypostome: Spatulate, rounded terminally. Dentition 2/2. Length about 0.11.

Scutum: Length 0.24, width 0.36. Very broadly rounded behind. Widest a little back of the middle. Cervical grooves shallow, nearly parallel. Surface smooth, shining, shagreened, impunctate.

Coxae: Spurs faintly visible. Each coxa with a single short, triangular spur, that on I largest, those on II and III progressively smaller. Hairs absent.

DISTRIBUTION AND HOSTS

The type was from "Carolina"; the type host is unknown.

In the United States the species is apparently established only in the areas bordering the Gulf of Mexico, and along the Atlantic Coast. Bishopp and Hixson (1936) state that this tick is seldom found in numbers more than 100 miles inland and that its distribution suggests "that rather high rainfall, humidity and temperature are necessary to its existence." Early records of *A. maculatum* from outside the present known normal limits of distribution are as follows: from Virginia (Niles, 1898, as *Dermacentor occidentalis*); from Memphis, Tenn. (Hunter and Hooker, 1907); from Tulare County, Calif. (Banks, 1908); from Dallas, Texas (Hooker et al, 1912). We have two recent records in the same category, i.e., a female from unknown host, Willcox, Arizona, Sept. 22, 1942 (L. P. Wehrle) and an engorged female from a dog, Weathers, Okla., Sept. 11, 1942 (Glen M. Kohls). Numerous records kindly supplied by Dr. Paul L. Piercy, Animal Parasitologist, Texas Agricultural Experiment Station, are entirely in keeping with those of this Laboratory and the map of Hooker et al (1912, p. 143). Neumann (1899, p. 252) records *A. maculatum* from Peru and Paraguay, and Neumann again (1911, p. 70) from Mexico, Ecuador, Chile and Patagonia. Robinson (1926, p. 44) records it from Brazil and Uruguay. Lahille (1905, pp. 152-153) reports that the species is common in Argentina. Newstead (1909, p. 445) reports males and females from Jamaica. Robinson (1926, p. 44) adds Colombia because of specimens donated by Dr. H. Gadow, and the Rocky Mountain Laboratory has several lots from Colombia sent by Dr. Luis Patiño-Camargo.

The adults attack a variety of the larger animals, both wild and domestic. Man is attacked occasionally. Larvae and nymphs attack birds principally but are found also on the smaller wild mammals. Hooker et al (1912) list as hosts of the adult ticks, cattle, sheep, horse, goat, dog, wolf and man, and of nymphs, meadow lark, red-winged blackbird, fox and jackrabbit. Bishopp (1912) listed collections of nymphs in southern Texas from meadow lark, *Sturnella magna*; red-winged blackbird, *Agelaius phoeniceus*; Brewer's blackbird, *Euphagus cyanocephalus*; and jack rabbit, *Lepus californicus merriami*. Hixson (Bishopp and Hixson, 1936, and Hixson, 1940), reporting from the vicinity of Valdosta, Ga., found the meadow lark to be the most important host of the immature forms during the fall and winter months. Larval hosts listed were meadow lark, *Sturnella magna*; bobwhite, *Colinus virginianus*; southern fox squirrel, *Sciurus niger niger*; eastern cotton rat, *Sigmodon hispidus hispidus*; towhee, *Pipilo erythrophthalmus*; field sparrow, *Spizella pusilla pusilla*; southern gray squirrel, *Sciurus carolinensis carolinensis*; house wren, *Troglodytes aedon*; brown thrasher, *Toxostoma rufum*; kid; lamb; mocking bird, *Mimus polyglottos*; blue jay, *Cyanocitta cristata*; loggerhead shrike, *Lanius ludovicianus ludovicianus*; and roof rat, *Rattus rattus alexandrinus*. Listed as host of the nymph were meadow lark, bobwhite, eastern cotton rat, southern fox squirrel, sheep, calf, towhee, house wren, American robin, *Turdus migratorius*; and the white-throated sparrow, *Zonotrichia albicollis*. Meadow larks and bobwhites were regarded as the most im-

portant hosts. Peters (1936) recorded the Carolina wren, *Thryothorus ludovicianus*, and the Red-eyed Towhee, *Pipilo erythrophthalmus*, as hosts in South Carolina without mention of stage or stages found.

For hosts outside of the United States see Robinson (1926).

Following are the records for the United States of the Rocky Mountain Laboratory:

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
16004	Ala.	Auburn, Lee Co.	7/26/39	Cow	3 ♀	Dr. E. W. Price
19377	Fla.	Bombay, Holmes Co.	9/6/41	Ears of cattle	2 ♀	L. E. Swanson
17153	Ga.	Nashville, Berrien Co.	7/29/40	Sheep	192 A.	Mr. Van Wheless
17040	"	"	8/9/40	"	75 A.	"
19650	"	Savannah, Chatham Co.			5 A.	R. M. L.
16008	"	Albany, Dougherty Co.	8/14/39	Dogs	6 A.	Geo. G. Brigham
16658	"	Ludowici, Long Co.	7/12/39	Sheep	4 A.	Dr. E. W. Price
14172	"	"			100 A.	B. A. I.
20495	Miss.	Keesler Field, Harrison Co.	8/29/43	Man	1 ♂	F. N. Young, Jr.
14171	"	Jackson, Hinds Co.			50 A.	B. A. I.
14078	"	"		Cattle?	Many	Dr. E. W. Price
19933	Okla.	Weathers, Pittsburg Co.	9/11/42	6 dogs	1 ♀	R. M. L.
13935	Tex.	Tarleton, Prairie, Erath Co.	8/3/37	Cow	3 A.	"
19924	"	Galveston, Galveston Co.	8/27/42	Dogs	7 A.	Kohls and McGregor
20459	"	Saratoga, Hardin Co.			1 ♂	Southwest Biological Sup. Co.
17235	"	Beaumont, Jefferson Co.	9/3/40	Mule	1 A.	R. M. L.
17239	"	"	9/5/40	Horses, mules, cattle	200 A.	"
17241	"	"	9/6/40	Horses and cattle	85 A.	"
11437	Tex.	Kingsville, Kleberg Co.	10/17/35	Horse	7 A.	R. M. L.
11144	"	Riviera, Kleberg Co.	10/19/35	Dog	6 A.	"
11443	"	"	"	Horse	2 A.	"
14832	"	Kingsville, Kleberg Co.	7/18/38	Coyote	1 A.	J. C. Brown
13826	"	Liberty Co.	7/19/37	Cow	1 A.	St. Bd. Hlth.
13917	"	Cleveland, Liberty Co.	7/23/37	Dogs	2 A.	"
13930	"	"	8/4/37	Dog	2 ♀	R. M. L.
13850	"	"	8/9/37	12 cows	23 A.	"
13857	"	"	8/10/37	2 horses	1 ♀	"
13866	"	"	8/12/37	Cows	1 A.	"
14926	"	Dayton, Liberty Co.	8/9/38	13 dogs	23 A.	"
14927	"	"	"	12 cows	86 A.	"
14928	"	"	"	Horse	7 A.	"
14930	"	Cleveland, Liberty Co.	8/12/38	20 horses	40 A.	"
14929	"	Dayton, Liberty Co.	"	Drag	46 A.	"
14931	"	"	8/13/38	Drag	60 A.	"
14932	"	Cleveland, Liberty Co.	"	45 horses	62 A.	"
17228	"	Dayton, Liberty Co.	8/31/40	Drag	62 A.	"
17231	"	Dayton, Liberty Co.	9/2/40	Cattle and dogs	18 A.	R. M. L.
17234	"	"	"	Drag	48 A.	"
19891	"	"	7/24/42	Cattle	100 A.	T. McGregor
19954	"	"	8/15/42		192 A.	"
19953	"	Liberty, Liberty Co.	8/16/42		214 A.	"
17242	"	Inez, Victoria Co.	9/9/40	Cattle	75 A.	R. M. L.
17245	"	Victoria, Victoria Co.	"	"	85 A.	R. M. L.
17247	"	"	"	"	66 A.	"

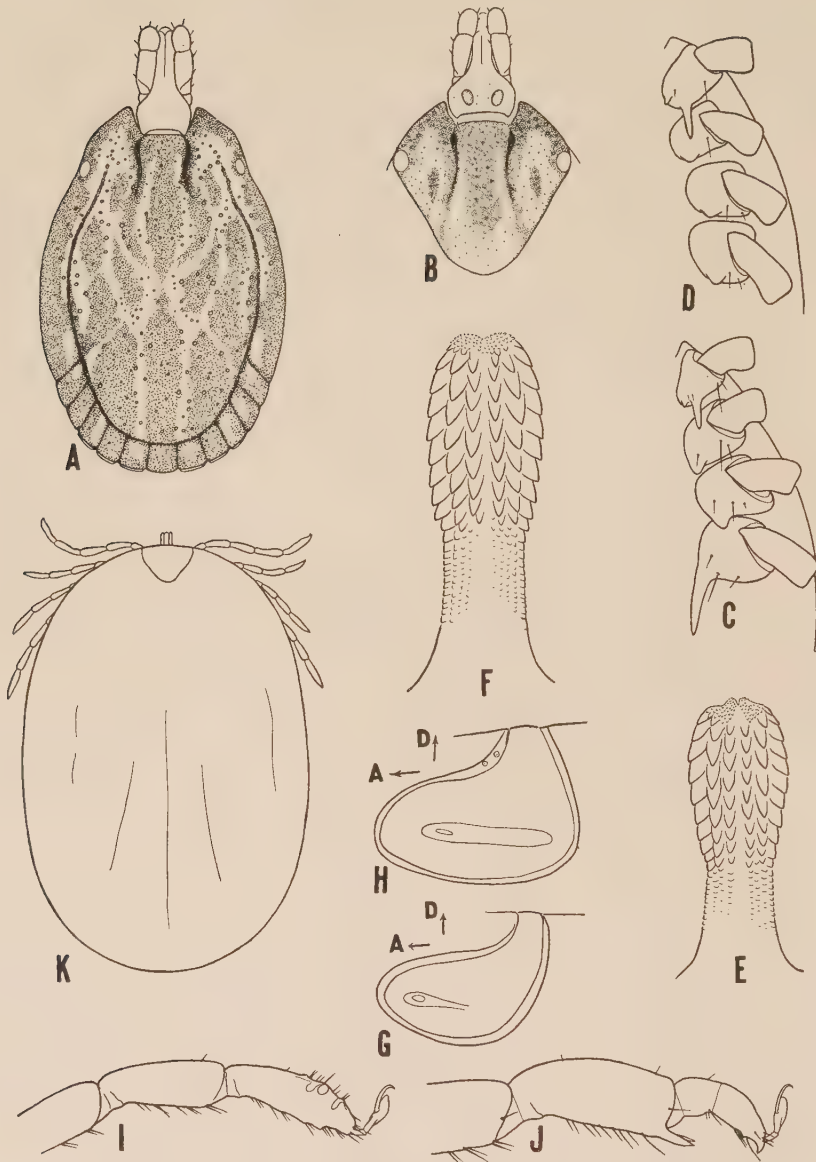


FIG. 5. *Amblyomma maculatum* Koch. A. Capitulum and scutum, male. B. Capitulum and scutum, female. C. Coxae of male. D. Coxae of female. E. Hypostome of male. F. Hypostome of female. G. Spiracular plate of male. H. Spiracular plate of female. I. Leg I of male. J. Leg IV of male. K. Engorged female.

Amblyomma dissimile Koch 1844
(Figs. 7 and 8)

- 1844. *Amblyomma dissimile* Koch, p. 225.
- 1844. *Amblyomma irroratum* Koch, p. 225.
- 1844. *Amblyomma adspersum* Koch, p. 226.
- 1844. *Amblyomma infumatum* Koch, p. 228.
- 1844. *Ixodes flavidus* Koch, p. 233.
- 1844. *Ixodes humanus* Koch, p. 233.

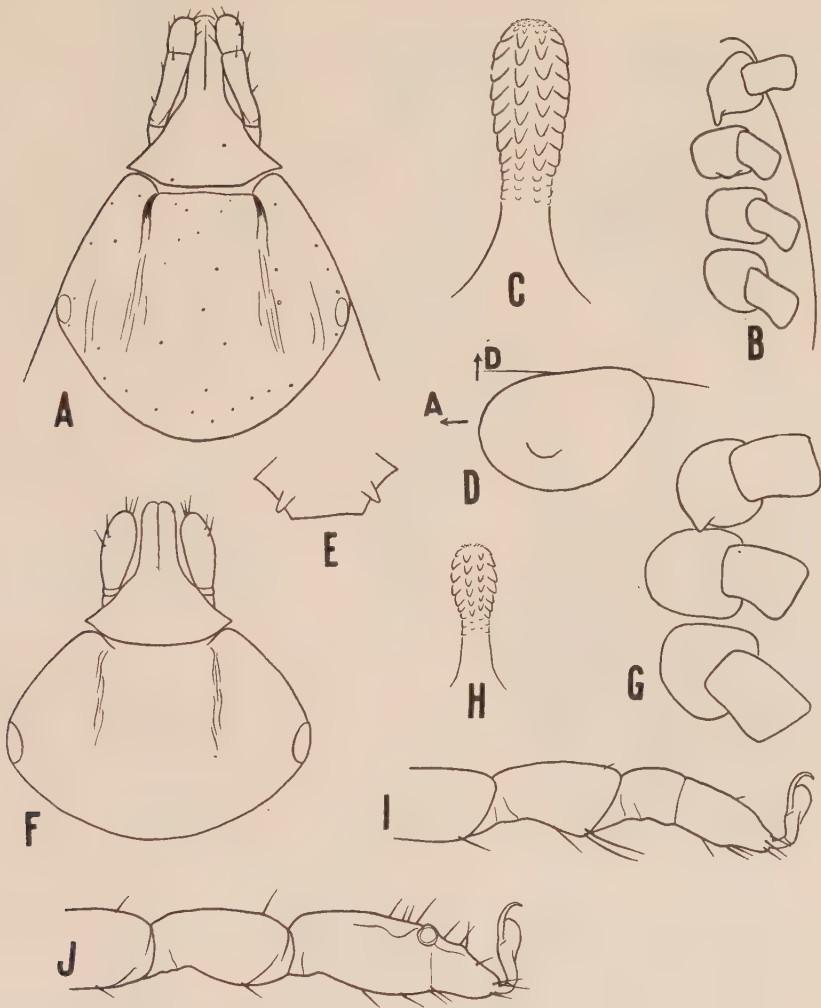


FIG. 6. *Amblyomma maculatum* Koch. A. Capitulum and scutum of nymph. B. Coxae of nymph. C. Hypostome of nymph. D. Spiracular plate of nymph. E. Spurs on venter of nymphal basis capituli. F. Capitulum and scutum of larva. G. Coxae of larva. H. Hypostome of larva. I. Leg IV of nymph. J. Leg I of larva.

1846. *Ixodes pulchellus* Lucas, p. 61.
 1886-1893. *Ixodes boarum* Stoll, p. 18.
 1911. *Amblyomma dissimile* Koch: Neumann, p. 72.
 1912. *Amblyomma dissimile* Koch: Hooker, Bishopp and Wood, p. 130.
 1926. *Amblyomma dissimile* Koch: Robinson, p. 163.
 1936. *Amblyomma dissimile* Koch: Oudemans, p. 471.

Female

Body: Unengorged body about 3.6 long by 3.0 wide; moderately well sclerotized. Much engorged body, 15.00 long by 9.00 wide; wider posteriorly.

Scutum: Length from 1.74 to 2.28; width from 2.10 to 2.58. Robinson (1926) gives size as 2.45×2.85 . Sub-triangular, a little wider than long, postero-lateral sides nearly straight. Cervical grooves sigmoid-shaped, deep anteriorly, fading out behind. Punctations few, large and small ones intermingled. Ornate, with irregular spots in antero-lateral areas, and a large, pale spot in the median posterior area. Eyes large, flat, pale.

Capitulum: Length 1.14 to 1.41, width of basis 0.75 to 0.87. Robinson (1926) gives length as 1.6. Sub-rectangular, lateral margins convex. Surface sparingly punctate. Porose areas large,

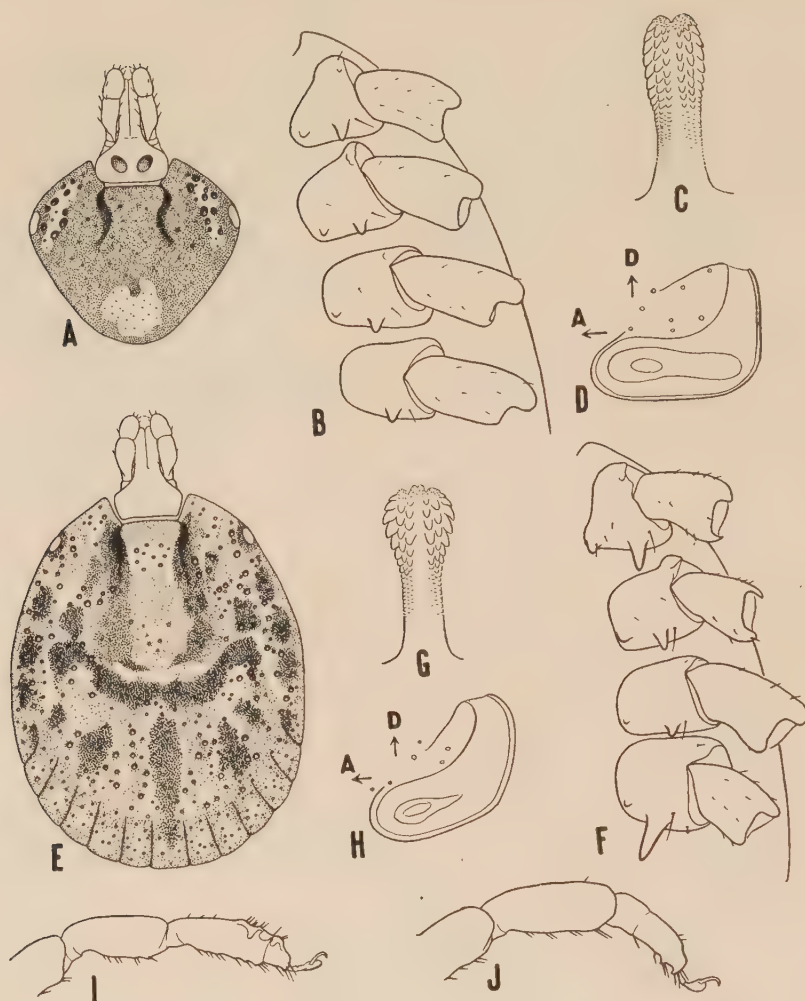


FIG. 7. *Amblyomma dissimile* Koch. A. Scutum and capitulum of female. B. Coxae of female. C. Hypostome of female. D. Spiracular plate of female. E. Capitulum and scutum of male. F. Coxae of male. G. Hypostome of male. H. Spiracular plate of male. I. Leg I of male. J. Leg IV of male.

oval, divergent anteriorly, and separated by about the shorter axis of one. Palpi long, clavate, laterally compressed; combined length of 2 and 3, 0.78 to 0.96.

Hypostome: Long, spatulate, notched apically. Dentition 3/3. Length about 0.69.

Legs: Of medium length, slender, smooth. Apical ventral spur faint on tarsus I, distinct on II, III and IV. Sub-apical ventral spur absent on I; small on II, III and IV. Length of tarsus I, 0.93; metatarsus, 0.72. Length of tarsus IV, 0.75; metatarsus, 0.75.

Coxae: In unfed specimens, coxae situated distant from the lateral margins of the body. A short, external spur present on all coxae. Coxae I, II and III with very short internal spurs; absent on IV. Robinson (1926) describes and figures the internal spur as present on IV.

Spiracular plate: Large, heavily chitinated, surface depressed. Greatest length 0.78, greatest width 0.60.

Genital aperture: Situated between coxae II.

Male

Body: Length from 2.75 to 4.50, width from 2.35 to 3.75. Broad oval, a little wider behind. Disparity in sizes is notable and small specimens have the color pattern less distinct.

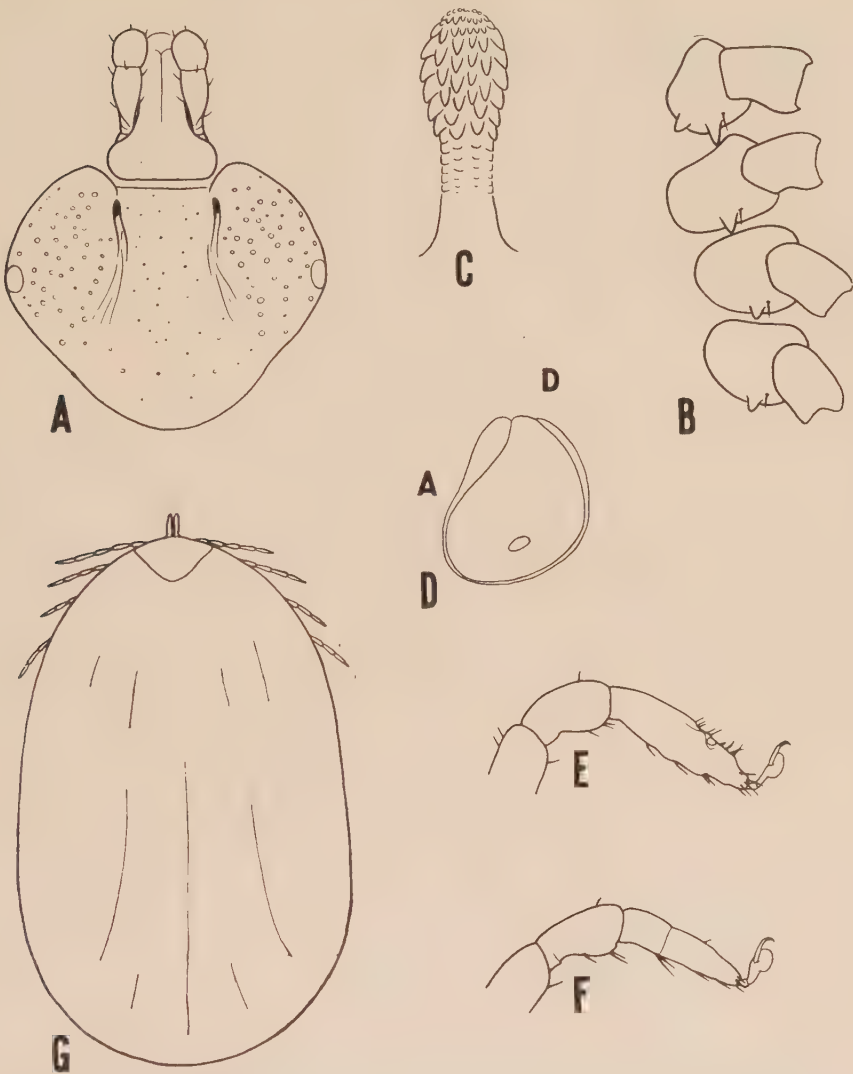


FIG. 8. *Amblyomma dissimile* Koch. A. Capitulum and scutum of nymph. B. Coxae of nymph. C. Hypostome of nymph. D. Spiracular plate of nymph. E. Leg I of nymph. F. Leg IV of nymph. G. Engorged female.

Scutum: Smooth, mildly convex, declivitous at sides and posteriorly. Lateral grooves absent. Cervical grooves deep and short. Ornate, with reddish-brown stripes on a pale yellow pattern tinged with rose-red. Pattern shown in the figure. Punctations large and small, intermingled, numerous in both light and dark spots. Fестоons long.

Capitulum: Length from 0.84 to 1.32; width of basis from 0.60 to 0.87. Essentially as in the female. Combined length of palpal articles 2 and 3 from 0.57 to 0.84.

Hypostome: Long, spatulate, faintly notched and sinuous apically. Dentition 3/3. Length about 0.63.

Legs: Essentially as in the female. Length of tarsus I, 0.84; metatarsus, 0.72. Length of tarsus IV, 0.75; metatarsus, 0.9.

Coxae: All coxae with two spurs, with external spurs larger. External spurs I and IV about equal, II and III equal but smaller. Internal spurs progressively smaller from I to IV, that on IV very small.

Spiracular plate: Large and heavily sclerotized. Greatest length 0.84; greatest width 0.45.

Genital aperture: Situated between coxae II.

Nymph

Well engorged specimens are 3.9 long and 2.94 wide. No unfed specimens are available.

Capitulum: Length 0.42, width of basis 0.3. Basis convex on top with the lateral profile margins curved. Posterior margin a little convex; postero-lateral corners faint. Palpi moderate in length; surface irregular. Length 0.3.

Scutum: Length 0.66, width 0.96. Much wider than long. Cervical grooves sigmoid, about half the length of the scutum; deep anteriorly. Surface faintly irregular. Punctations moderate in number, larger and deeper in the antero-lateral areas.

Hypostome: Spatulate, rounded apically. Dentition 3/3. Length about 0.87.

Legs: Ventral spurs on tarsi absent. Length of tarsus I, 0.39; metatarsus, 0.21. Length of tarsus IV, 0.33; metatarsus, 0.24.

Coxae: Coxa I with two small spurs; II, III and IV with one small spur.

Spiracular plate: Much less sclerotized than in the adults. Greatest length 0.24; width, 0.18.

DISTRIBUTION AND HOSTS

The only United States records of the species are those of Bequaert (1932) from southern Florida, i.e., from gopher snake, *Spilotes corais couperi* (Holbrook), Sebastian, Indian River Co., February, 1909, and from pigmy or ground rattler, *Sistrurus miliaris* (Linnaeus), Boca Ratone, Palm Beach Co. (1931).

The species has an extended southern range which, according to Bequaert, includes Mexico, Guatemala, British Honduras, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Venezuela, Trinidad, Grenada, Tobago, British Guiana, Peru and Brazil.

Known hosts include 20 different kinds of Reptilia and Amphibia. In addition to these, Robinson (1926) includes a record from cow and from sheep, and Neumann (1911) mentions the capybara, "*Hydrochoerus hydrochoeris* (L.) (*H. capybara* Erxl.)."

Amblyomma tuberculatum Marx, 1894

(Figs. 9 and 10)

1894. *Amblyomma tuberculatum* Marx, p. 314.

1908. *Amblyomma tuberculatum* Marx: Banks, p. 38.

1911. *Amblyomma tuberculatum* Marx: Neumann, p. 74.

1912. *Amblyomma tuberculatum* Marx: Hooker, Bishopp and Wood, p. 123.

1926. *Amblyomma tuberculatum* Marx: Robinson, p. 174.

1932. *Amblyomma tuberculatum* Marx: Bequaert, p. 778.

Female

Body: The largest known species of the genus in the United States. Length 7.0 to 7.5, width 5.5 to 6.00. Broad oval, heavily sclerotized. Scutum reaching about two-fifths the length of body.

Scutum: Length 3.6, width 4.2. Shape broadly cordiform. Cervical grooves deep and curved. Ornate with dark-brown markings on a yellow-white ground. Pattern shown in the figure. Fine punctations very numerous and with a few large ones in the antero-lateral areas. Eyes small, flat, pale.

Capitulum: Length 2.3 to 2.60, width of basis 1.56. Basis sub-rectangular, posterior margin straight. Porose areas small, oval, divergent anteriorly, widely separated. Palpi long, relatively longer than those in the male. Combined length of 2 and 3, 1.59.

Hypostome: Spatulate, long, with the denticles 4/4, occupying two-fifths the total length. Length about 1.08.

Legs: Apical ventral spurs present on all the tarsi. Sub-apical ventral spurs present on tarsi II, III and IV. Length of tarsus I, 1.14; metatarsus, 1.10. Length of tarsus IV, 1.20; metatarsus, 1.53.

Coxae: Coxae I to IV each with two short, broad, flat spurs; internal spur on IV smaller.

Spiracular plate: Large, heavily chitinated, with the surface much depressed.

Genital aperture: Opposite the posterior borders of coxae II.

Male

Body: Length 6.2, width 5.5. Broad oval, wider behind, sides evenly rounded.

Scutum: Smooth, convex, all margins declivitous. Cervical grooves long, curved, deep in their anterior ends. Marginal grooves absent. Ornate, with dark-brown spots, mainly separated, on a dull, yellow-white ground. Pattern shown in the figure. Punctations very numerous and fine, and with a few large ones intermingled. Festoons short; ventral scutes protruding farther than the festoons, leaving narrow shelves visible from above.

Capitulum: Length 1.8, width of basis 1.23. Essentially as in the female but with the palpi relatively shorter. Combined length of palpal articles 2 and 3, 1.05.

Hypostome: Spatulate, faintly notched apically. Denticles 4/4. Length about 1.20.

Legs: Apical ventral and sub-apical spurs present on all tarsi. Length of tarsus I, 0.93; metatarsus, 0.93. Length of tarsus IV, 0.84; metatarsus, 1.50.

Coxae: Coxae I to IV each with two short, broad, flat spurs. Internal spur on IV smaller and situated on the median edge of the coxa.

Genital aperture: Situated between coxae II.

Nymph

Capitulum: Length 0.93, width of basis 0.66. Basis sub-quadrate, convex, with sides not marginated but merging into the lateral walls. Posterior margin nearly straight. Palpi long, widest on article 3.

Scutum: Length 1.44, width 1.74. Broadly cordiform with the postero-lateral margins a little flattened. Cervical grooves curved and deep. Ornate, with dark brown markings on dark yellowish-white ground. Pattern shown in the figure. Surface very finely pebbled. Large, deep punctations numerous in the lateral areas, small punctations in the median areas.

Hypostome: Shape as in the adults. Dentition 3/3. Length about 0.36.

Legs: All tarsi with the apical, ventral spur present as in adults, but small. Length of tarsus I, 0.60; metatarsus, 0.45. Length of tarsus IV, 0.60; metatarsus, 0.63.

Coxae: Coxae I and II each with two broad, short, flat spurs; internal spurs smaller. Coxae III and IV each with one external spur; internal spurs lacking.

Spiracular plate: Large and heavily sclerotized. Surface much depressed. Length and width about equal—0.33.

Larva

From cast skins of fully fed larvae we are able to describe the larva in part.

Scutum: Length 0.45, width 0.57. Cervical grooves long, extending about three-fourths the length of the scutum, deeper anteriorly. Surface impunctate.

Capitulum: Sub-triangular with rounded points at the sides. Length 0.312, width of basis 0.221. Combined length of articles 2 and 3, 0.18.

Hypostome: Dentition 2/2. Length 0.192.

Coxae: Coxa I with two small spurs; II and III each with one small spur.

DISTRIBUTION AND HOSTS

This species is known in this country only from Alabama, Georgia and Florida. Bequaert (1932) stated:

A. tuberculatum is, in the adult and nymphal stages, a common and specific parasite of the gopher turtle, *Gopherus polyphemus* (Daudin). Most probably its range coincides with that of the host, which occurs throughout the coastal plain of the Southeastern United States. L. Stejneger and T. Barbour (1923, A Check List of North American Amphibians and Reptiles, 2d ed., p. 137) give the distribution of this gopher turtle as "Coast from southern South Carolina to Florida and the Mississippi River north into southern Arkansas." Dr. Barbour tells me that it does not occur in the extreme southern part of Florida and that it certainly was never indigenous in Cuba. Neumann (1899) recorded a male of *A. tuberculatum*, at the Paris Museum, collected by Gundlach in Cuba; but this specimen must have been taken off a gopher turtle brought from the mainland and kept in captivity.

Previous, definite locality records of *A. tuberculatum* are as follows: Crescent City, Putnam Co., Florida (type locality; Marx and Hubbard, 1894). Enterprise, Volusia Co., Florida (Banks, 1904). Hawthorn, Alachua Co., Florida (Hooker, Bishopp and Wood, 1912). Southern Alabama (Hooker, 1909; Robinson, 1926).

I have seen five different lots of this tick, all taken off *Gopherus polyphemus*, from the following localities: Lakeland, Polk Co., Florida (Am. M. N. H. and W. T. Davis Coll.). La Grange, Brevard Co., Florida (W. T. Davis Coll.). Mt. Pleasant, Gadsden Co., Florida (W. T. Davis Coll.). South Carolina, without more definite locality (off a turtle brought to the Bronx Zoological Park.—W. T. Davis Coll.).

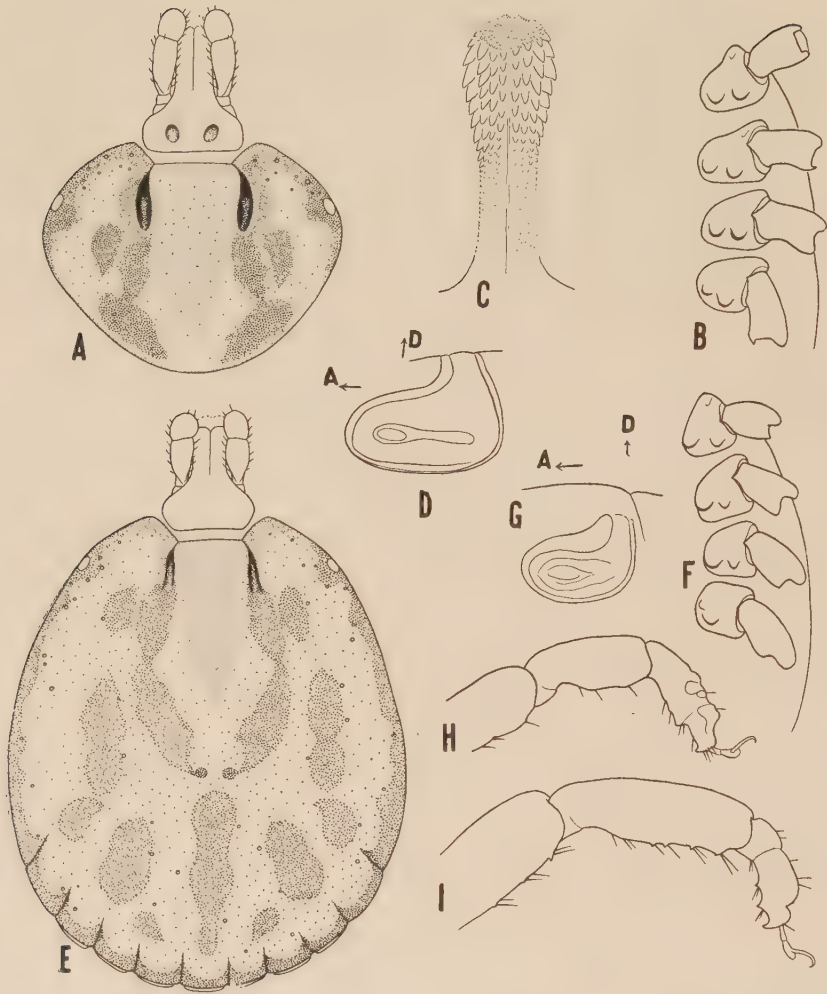


FIG. 9. *Amblyomma tuberculatum* Marx. A. Capitulum and scutum of female. B. Coxae of female. C. Hypostome of female. D. Spiracular plate of female. E. Capitulum and scutum of male. F. Coxae of male. G. Spiracular plate of male. H. Leg I of male. I. Leg IV of female.

The southern meadowlark, *Sturnella magna argutula*, has been recorded as a host in Florida, without mention of the stage of the tick.

Hooker, Bishopp and Wood (1912) stated, "engorged larvae have been collected in large numbers from dogs and rabbits and in smaller numbers from cattle and two birds of prey, namely, the owl and the hawk."

Young and Goff (1939) give the following records, all from *Gopherus polyphemus* in Florida: Miami, May 20, 1933; Leesburg, May 4, 1938, and Gainesville, November 28, 1936.

The Rocky Mountain Laboratory collection contains specimens as follows: 18545, *Gopherus polyphemus*, Florida, April 23, 1939, 4 ♂ (Amer. Mus. Nat. History); 19833, *Sceloporus undulatus undulatus*, Silver Springs, Marion Co., Florida, February 28, 1942, 5 nymphs (J. Frenkel); 16266, *Geomys floridanus*, Newman's Lake, Alachua Co., Florida, December 9, 1939, several larvae (B. A. Barrington) and

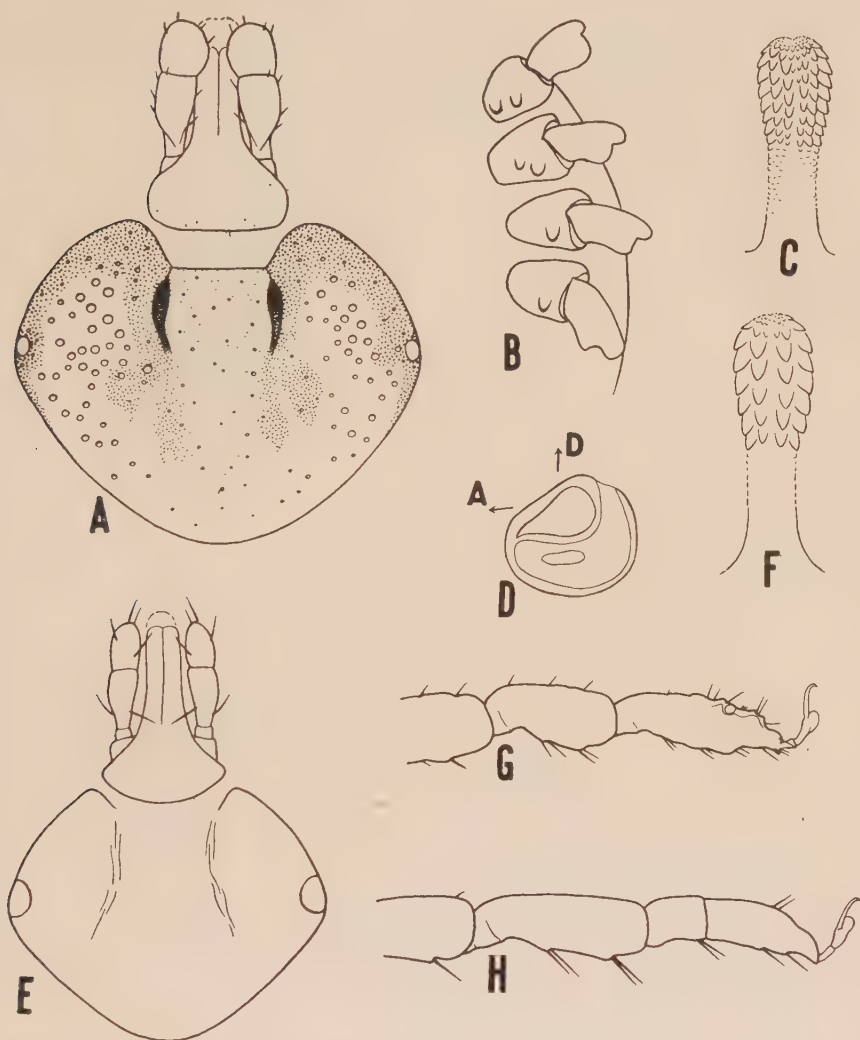


FIG. 10. *Amblyomma tuberculatum* Marx. A. Capitulum and scutum of ornate nymph. B. Coxae of nymph. C. Hypostome of nymph. D. Spiracular plate of nymph. E. Capitulum and scutum of larva. F. Hypostome of larva. G. Leg I of nymph. H. Leg IV of nymph.

19651, Broxton, Coffee Co., Georgia, host and date unknown, 1 ♂, 1 ♀ (Dr. G. Brigham).

Amblyomma inornatum (Banks, 1909)

(Fig. 11)

1909. *Aponomma inornata* Banks, p. 171.

1939. *Amblyomma philipi* Cooley and Kohls, p. 44.

Female

Body: Banks (1909) in describing the species gives the length as 8 mm. We have neither unengorged nor fully engorged specimens. One slightly fed specimen is oval, narrower in front.

Scutum: Length 1.36 to 1.62, width 1.44 to 1.56. Inornate. About as long as wide, widest a little in front of the middle. Broadly rounded behind, with the postero-lateral margins a little convex. Cervical grooves deep in front, then continuing as valleys and disappearing without reaching the postero-lateral margins. Punctations numerous, moderate in size. Some specimens have smooth elevations without punctations near the eyes which suggest lateral carinae. Eyes large, pale, slightly convex and margined by small punctations.

Capitulum: Length 0.90 to 1.02, width of basis 0.57 to 0.66. Basis sub-triangular, with the posterior margin straight, postero-lateral edges salient. Porose areas large, oval, divergent anteriorly and well separated. Surface smooth, shining, punctate. Palpi long, narrow proximally. Postero-dorsal ridge moderate. Hairs moderate in number.

Hypostome: Long, spatulate, faintly notched apically. Dentition 3/3. Length about 0.54.

Legs: Moderate in length and size. Apical ventral spur absent on tarsus I, present on II, III and IV. Sub-apical ventral spurs absent. Length of tarsus I, 0.54; metatarsus, 0.36. Length of tarsus IV, 0.48; metatarsus, 0.42.

Coxae: Coxa I with a short internal and a moderately long external spur. Coxae II, III and IV each with one broad, triangular, external spur. Faint trochantal spurs present on legs I and II. All coxae with a few hairs.

Spiracular plate: Moderate in size, flat, well sclerotized. Length 0.45, width 0.36.

Genital aperture: Situated between coxae II.

Male

Body: Length 1.86 to 2.04, width 1.32 to 1.44. Shape almost pyriform.

Scutum: Inornate. Smooth and shining. Lateral grooves distinct, starting back of the eyes and limiting the long festoons. Cervical grooves deep and short. Eyes small, pale and only slightly convex. Posterior median surface in front of the festoons more elevated than the festoons. Punctations numerous, large and present also on the festoons. Hairs absent.

Capitulum: Length 0.54 to 0.60, width of basis 0.37 to 0.39. Basis sub-triangular, cornua a little pointed, posterior margin between the cornua nearly straight, salient. Surface smooth, shining, punctate. Hairs absent. Palpi moderate in length, much broader distally, smooth, shining, impunctate, and with a few short hairs.

Hypostome: Spatulate, broad distally, very faintly notched apically. Dentition 3/3. Length about 0.3.

Legs: Terminal ventral spur absent on tarsus I, present on II, III and IV. Sub-apical ventral spurs absent. Length of tarsus I, 0.48; metatarsus, 0.30. Length of tarsus IV, 0.42; metatarsus, 0.375.

Coxae: All coxae as in the female. Faint trochantal spurs present on I and II in some specimens.

Spiracular plate: Large, flat, moderately sclerotized. Length 0.48, width 0.195.

Genital aperture: Situated between coxae II.

A. inornatum rather closely resembles *A. parvum* Aragão, 1908. *A. inornatum* is smaller, lacks the retrograde spur on the venter of the male palpal article 1, which in the female is poorly developed, and has the much larger punctations on the scuta of both sexes. In the males the most notable differences are in the palpi in dorsal view. In *parvum* the palpus is short and broad, and on article 2 has the postero-dorsal ridge prominent and sometimes overhanging.

HOSTS AND DISTRIBUTION

Amblyomma inornatum was described from dog at Corpus Christi, Texas, and from the rabbit, Victoria, Texas. No type specimens were so labelled but Dr. Bishopp has shown the senior author specimens believed to be the ones which Banks had before him when the species was described. The above descriptions are based on specimens in the collections of the Rocky Mountain Laboratory taken at Kingsville, Texas.

All other known records are tabulated below.

Accession No.	State	Locality	Date	Host animal or source	Number	Collector or authority
14069	Texas	Kingsville	6-10-38	Rabbit	1 ♀	J. C. Brown
14190	"	"	3-24-38	Rabbit	1 ♂	"
14295	"	"	5-24-38	Jackrabbit	1 ♂	R. M. L.
14329	"	"	5-25-38	<i>Canis</i> sp. (coyote)	4 ♂, 1 ♀	R. M. L.
14330	"	"				
14331	"	"				
14332	"	"				
17247	"	27 mi. N.E. of Victoria	9-9-40	Cow	2 ♀	"

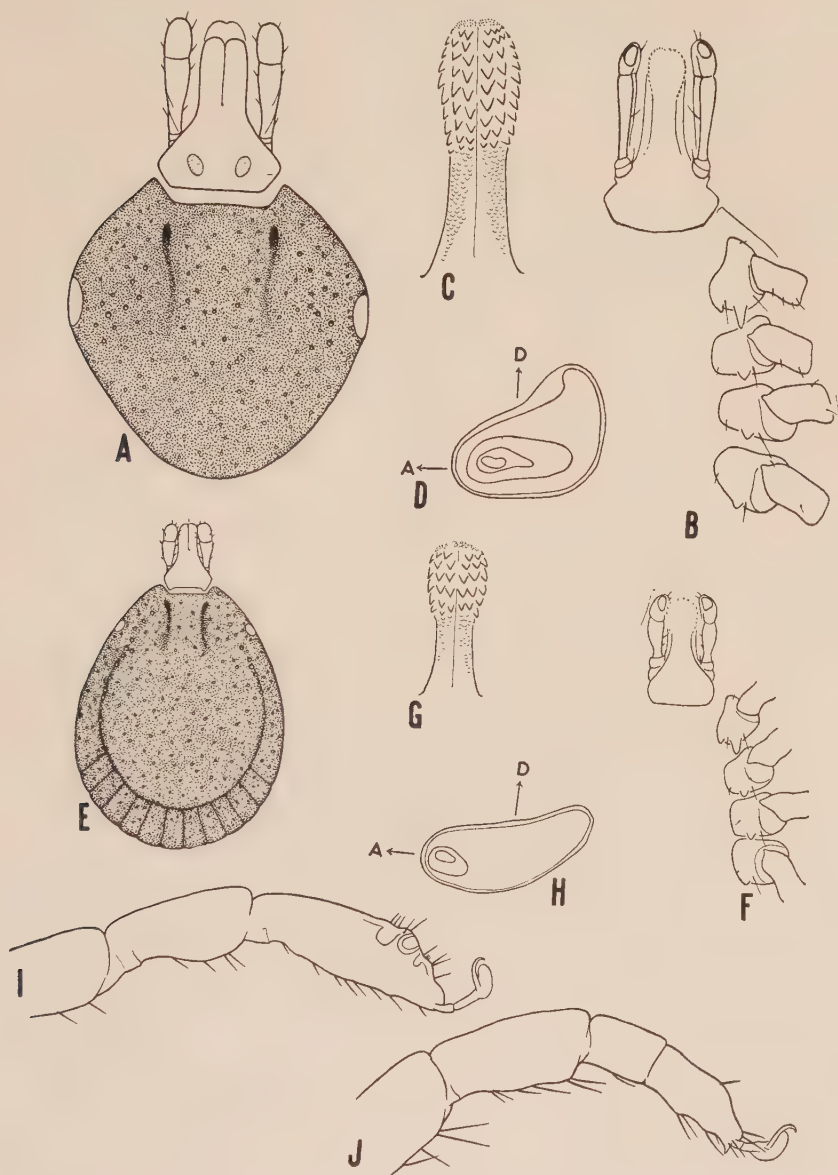


FIG. 11. *Amblyomma inornatum* (Banks). A. Capitulum and scutum of female. B. Capitulum (venter) and coxae of female. C. Hypostome of female. D. Spiracular plate of female. E. Capitulum and scutum of male. F. Capitulum (venter) and coxae of male. G. Hypostome of male. H. Spiracular plate of male. I. Leg I of female. J. Leg IV of female.

Amblyomma avecolens n. sp.

(Fig. 12)

Male and female unknown.

Nymph

Body: No unengorged specimens are available. Well-engorged specimens are very large, long-oval, slightly wider in front, measuring 6.5 long by 4.0.

Scutum: Length 1.02 to 1.14, width 1.08 to 1.11. Widest at the middle, scapulae pointed. Cervical grooves divergent backward, deep in front, and become valleys. Punctations conspicuous, deep, evenly distributed and contrasting strongly with the finely pebbled surface. Eyes small and flat.

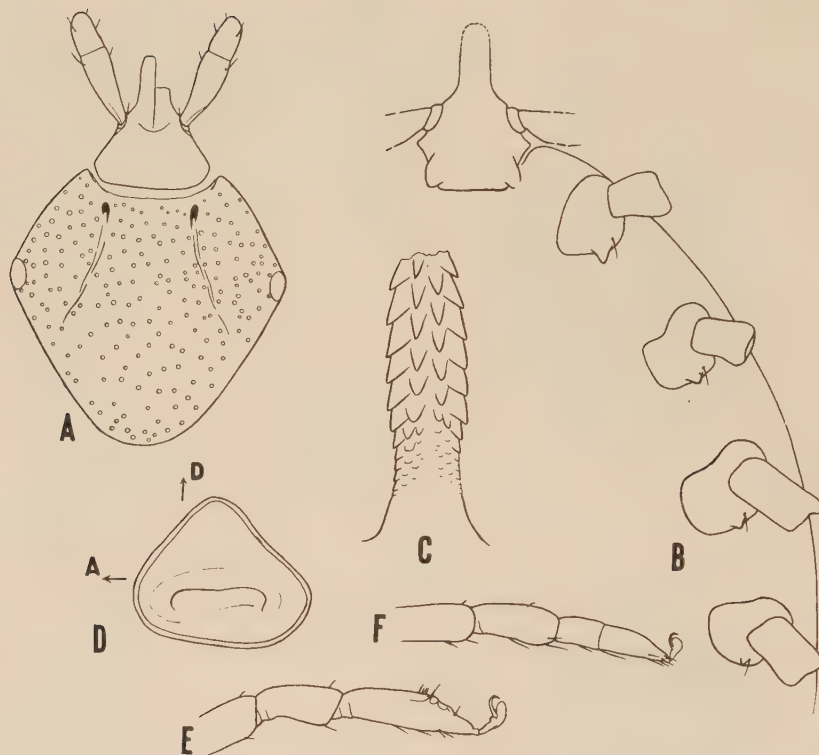


FIG. 12. *Amblyomma avecolens* n. sp. A. Capitulum and scutum of nymph. B. Capitulum (venter) and coxae of nymph. C. Hypostome of nymph. D. Spiracular plate of nymph. E. Leg I of nymph. F. Leg IV of nymph.

Capitulum: width of basis 0.45 to 0.48. (All capituli are mutilated and the length cannot be determined.) Sub-triangular, broadly rounded behind. Surface of basis and palpi finely pebbled, similar to the scutum. In ventral view basis is broad, with low lateral retrograde projections at the sides. Palpi long with a few fine hairs.

Hypostome: Long, with sides about parallel (the tip broken off). Dentition 2/2 and with files extending to near the base. Length (incomplete) 0.36.

Legs: Surface not smooth, suggesting the surface of the palpi. Tarsi long and tapering gradually; apical-ventral and sub-apical ventral spurs absent on all tarsi. Hairs few and short. Length of tarsus I, 0.54; metatarsus, 0.36. Length of tarsus IV, 0.45; metatarsus, 0.36.

Coxae: Small and with small, external spurs progressively smaller from I to IV, those on III and IV seen only with difficulty.

Spiracular plate: Sub-triangular, with the surface smooth, except at the macula, which is much depressed. Size 0.27 by 0.27.

Through the kindness of Dr. E. A. Chapin, Curator, Division of Insects, of the United States National Museum, we have had the opportunity to study three lots of nymphal ticks from birds, as listed below. The scutum is very characteristic and the bird host labels are of interest. These specimens are not easily referred to genus, but it appears reasonably certain that they are of a species of *Amblyomma*. The early stages of *Amblyomma* are not well known. Robinson (1926) omits them. Some of the specimens here described are very large and could not be of any known species in the United States, and the species of *Amblyomma* in Mexico are very little known.

Holotype: Nymph, 18707,* 1 from neck of *Vireo griseus*, Aug. 5, 1929, 6 miles east of Sorita, Kennedy Co., Texas, deposited in the collections of the Rocky Mountain Laboratory.

* Numerals refer to records of the Rocky Mountain Laboratory.

Paratypes: 18709, 1 from *Glyphorynchus curvata*, May 3, 1926, Duck Run, British Honduras; 18702, 2 from *Xiphorynchus* sp., Mar. 24, 1912, Panama Canal, deposited in the United States National Museum.

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NOTES ON THE PUPAL DEVELOPMENT OF *STILBOMETOPA IMPRESSA* (DIPTERA: HIPPOBOSCIDAE)

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Stilbometopa impressa (Bigot), a common blood-sucking parasite of quail in California, has been reported only from *Lophortyx* spp. The present report is based on material collected entirely from *Lophortyx californica vallicola* and deals primarily with pupal development of the fly.

Knowledge of the biology of *Stilbometopa impressa* is of importance because of the fly's probable relationship to *Haemoproteus lophortyx*, a common malarial parasite of California quail. Thus far only the smaller fly *Lynchia hirsuta* Ferris has been demonstrated (O'Roke, 1930) to be a vector of this malaria. Work is now in progress in our laboratory to determine whether *S. impressa* is a vector of the malaria and as important in this regard as *L. hirsuta*.

HISTORY

Studies on the biology of the bird hippoboscids received greater impetus when Sergeant and Sergeant (1906) demonstrated *Pseudolynchia canariensis* (Macquart) (= *Lynchia maura*) to be the transmitting host of *Haemoproteus columbae* of domestic pigeons. Their findings have been confirmed subsequently by many investigators in various parts of the world. O'Roke (1930) showed the fly, *Lynchia hirsuta* to be a vector of *Haemoproteus lophortyx*, and Herms and Kadner (1937) utilized *Lynchia fusca* as a vector of the quail haemoproteid. No other hippoboscid fly has been demonstrated as a transmitting agent of the various species of *Haemoproteus* in birds.

The biology of only a few bird hippoboscids has been investigated and *P. canariensis* has received the most attention. Sergeant and Sergeant (1907) stated that the larva, when deposited, is ovoid in shape, white, except at one pole, which is covered with a small black area containing six distinct branches. It becomes entirely black in one hour. These authors remarked that under the microscope very fine "hexagonal" lines appear on the surface of the pupa. Bishopp (1929) observed that the pupae of these flies are broadest posteriorly and slightly flattened dorso-ventrally. The surface is shiny and sculptured with "zigzag" lines except for the thickened posterior portion which is densely punctured and without lines. Drake and Jones (1930) reported that a single puparium of *P. canariensis* went through successive changes of yellow, light to dark brown and in the course of two or three hours, became strongly indurated, somewhat shiny and jet black in color. Coatney (1931) stated that the female fly deposits a single mature, whitish larva bearing a black post-abdominal spiracular plate and observed the same color changes of the puparium.

Huzimatu (1938) noted that the full grown larva of *Stenopteryx nipponica* Kishida, a parasite of martins in Japan, is globular in form and milky white in color. When the larva is deposited it is light brown in color, except the spiracular plate which is shining black. The integument afterwards becomes chitinated to form the

pupal case and there is a gradual darkening of the color until the puparium at last becomes shining black. From his observations (on two occasions) the color of the puparium turned from light brown to black in about two hours after deposition. He observed also, under the microscope, a network of fine lines.

In reporting the duration of pupal development, from the time the mature larva or pupa is deposited until the fly emerges, Sergeant and Sergeant (1907) in North Africa recorded 23 to 27 days (at a temperature of 42° C, which is the temperature of the plumage of the pigeon host); Adie (1915) in India, 31 to 36 days (but noted that some took 52 to 64 days to complete the pupal stage, and even longer as cold weather approached); Bedford (1924) in South Africa, 23 to 28 days; Bishopp, United States, 29 to 31 days (when the mean daily temperature was about 73° F—during the warmer part of the year development was accelerated slightly and the pupal period was longer under lower temperatures); Drake and Jones (1930), Iowa, (one larva deposited in September) 35 days; Coatney, also Iowa, 25 to 31 days (when kept at laboratory temperature—if the temperature was higher, the time was shortened a few days). Huzimatu reported that a single pupa of *Stenopteryx nipponica* produced an imago in 23 days. O'Roke observed that a single pupa of *Lynchia hirsuta* produced a fly in 31 days.

Hardenberg (1929) collected a number of *Stenopteryx hirundinis* Leach from swallows in Germany. He stated that the flies over-winter in the pupal state in the nest of the bird. He considered that there were two types of pupae: "summer" pupae which hatch out in about 22 days and "winter" pupae which do not hatch until the following year, not earlier than in April or May and usually not until the middle of June. Similar to Hardenberg's observations, Huzimatu noted pupae of *S. nipponica* in the abandoned nests of the birds after they had migrated southward and concluded that the imagoes would emerge in the spring of the next year when the birds came back to their old nests, thus also recognizing a distinction between summer and winter pupae.

Coatney observed that if the larva is tampered with before it has entirely pupated, in most cases it will fail to yield an imago. In observations on more than 50 larvae which had been handled prematurely, only one yielded an imago; 22 of these pupal cases were opened for examination and only two showed development which had been arrested early. Coatney found also that the flies usually deposited between 7 and 11 AM, seldom later in the day.

Coatney reported that of 221 imagoes of *P. canariensis* bred in the laboratory, 96 were males and 125 were females, concluding that the sexes are produced in about equal numbers. Hardenberg, in a series of 42 newly emerged *S. hirundinis*, counted 22 males and 20 females.

O'Roke, in his observations on *L. hirsuta* of quail in California, stated that no pupae or flies were taken apart from their hosts and no pupae were collected except those that were "oviposited"¹ in vials by captive flies.

Flies were taken in every month from June to October and hunters reported seeing *L. hirsuta* on quail during December and January.

¹ Undoubtedly means deposition of larva or pupa. These flies usually deposit their offspring in the pupal stage but may deposit them in the last phases of larval existence. Hence the deposited body may be referred to as a larva or pupa as the case may be.

MATERIALS AND METHODS

Flies were collected in the field primarily by Charles Edmondson, John Laughlin and David M. Selleck of the California Division of Fish and Game and Roy W. Saarni of the U. S. Forest Service. The flies were obtained mainly from trapped wild quail (*Lophortyx californica vallicola*) in Orange, Riverside and San Benito Counties and from the San Joaquin Experimental Range at O'Neals, Madera County. In all these areas *Stilbometopa impressa* is a common parasite; a few specimens of *Lynchia hirsuta* were collected in Orange County in August, 1941, but none was observed on the birds from the other three localities.

Some of the flies were brought to the laboratory in a small screened cage containing a single quail. The cage containing the bird was taken into the field during trapping operations and all flies captured from quail were immediately placed into the small cage where they could seek a haven on their natural host. Where it was not feasible to bring the flies to the laboratory by this method they were shipped in shell vials approximately $\frac{1}{2}$ inch in diameter and 2 inches deep. The aggressiveness of these flies made it necessary to ship only one per vial until Mr. Selleck experimented with placing a few quail feathers in each vial. Using this technique it was found that as many as 4 live flies could be shipped successfully. The feathers presumably served as a protection enabling the flies to escape each other's attacks. The flies were one or two days in transit.

As soon as the live flies were received at the laboratory they were placed in escape-proof cages with from one to seven quail. These cages were constructed with wire bottoms ($\frac{1}{2}$ -inch mesh) with a collecting pan one inch below.

Most of the flies were obtained in August and September but this is not to be interpreted as the time of greatest abundance of *Stilbometopa* on the quail. The birds were trapped during these months as a part of other projects; collection of the flies was only incidental to other research.

Observations on *S. impressa* were begun in 1941 at the laboratory of the Los Angeles Wildlife Disease Research Station of the U. S. Fish and Wildlife Service. Detailed data on the pupal period were not sought in the earlier studies. Pupae were collected from the experiment cages at irregular intervals, usually about fortnightly.

In the summer of 1942 the studies were resumed at the State Game Farm in Chino, California, and continued in the fall at the Fish and Game laboratory in San Francisco. The pans of the cages containing birds and flies were examined several times daily. Pupae, or larvae, were placed, individually, in shell vials of the same type described above, loosely plugged with cotton. They were examined hourly during the first day to note color changes and then daily until the flies emerged. Throughout these experiments in the laboratory no attempt was made to regulate temperature which, on the average, was approximately 70° F.

LIVABILITY OF ADULT FLIES

In the preliminary studies conducted in 1941 it was found that a number of flies placed in a cage with one or two quail yielded many pupae while an equal number of flies placed in a cage with seven quail produced very few, if any, pupae. Upon observation it was found that the birds would eat the flies. It was therefore assumed that in the limited confines of the cage with only one or two quail the flies were able

to avoid capture by the quail, while with many birds they had little chance to escape. The flies momentarily leave their hosts whenever the birds become excited, thus exposing themselves to capture. To test these points 25 flies were placed in an escape-proof cage with seven quail. Twenty-four hours later the birds were carefully removed and after extensive examination only a single fly was recovered. Similarly, 18 flies were placed in a cage with two quail. At examination three days later, 17 flies were recovered.

A colony of *S. impressa* was started by placing 47 adult flies (obtained from the wild quail) in an escape-proof cage with two quail. The flies were placed in the cage during August and September, 1941. Sixty-three pupae were collected at irregular intervals and of the 44 flies that emerged all, except the last one, were replaced into the same cage. During the experiment a total of 19 dead flies were collected in the pan beneath the cage. The flies seemed to steadily decrease in numbers (eaten by the birds?) until on March 20, 1942, no further flies could be found. (The last fly had been added to the cage on March 7, 1942.) A single fly emerged from a pupa after this date on April 13, 1942.

The longest period a fly remained alive off its host was five days.

DEPOSITION OF LARVAE

A single female was observed in the process of larvipositing. She supported herself on the first two pairs of legs. With one of the third pair of legs on the dorsal side of the body and the other on the ventral, the abdomen was stroked posteriorly as the larva slowly emerged through the vaginal opening. The entire process lasted over two minutes. Prouty and Coatney (1934) have reported a similar observation for *Pseudolynchia canariensis*. However, the author has observed females taken from wild quail which, in the excitement of being caught, seemed to larviposit almost instantaneously. It is questionable whether the straining procedure is not the exception rather than the rule.

The larva described in the preceding paragraph was creamy white when deposited but began to darken within a few seconds. Four others were observed during the course of these experiments. The larvae are soft, delicate, ovoid in shape, 5.5×4.5 mm. At the posterior end is a black spiracular plate, 1 mm in diameter. They quickly darken, usually in less than one minute, although one was observed to remain white for at least seven minutes and since it eventually produced a fly it was considered within the limits of normal development.

All larvae in the experiment cages were found in the pan below the false bottom. The fly observed during larviposition was in the pan. Frequently female flies were observed in the pan. From this evidence it seems logical to assume that the flies normally leave the host to deposit the larvae.

In the laboratory there seemed to be no evidence of deposition of larvae by the flies at any particular time of day.

DEVELOPMENT OF PUPAE

From the preliminary studies on flies collected during 1941 it was evident that the puparium passes through several color phases before it becomes the characteristic shining black. In a number of cases careful observation was made of the time required for the pupal case to achieve its shining black color. Table 1 summarizes much of the data on the development of the pupae during the 1942 experiments.

variation than those collected at later dates, the variation increasing as winter progressed. The 5 pupae collected on January 26, 1942, produced flies on March 2, 3, 6, 13 and April 13.

From the flies collected off wild quail during 1942, 38 pupae were obtained, the first on August 25, 1942, the last on October 28, 1942. Only 19 flies emerged. In each case the exact date of deposition was noted.

The pupal development may last from 61 days (or less, as shown in the preliminary experiments) to as long as 162 days. The duration of this stage seems to increase (but in no definite progression) as the season advances. There is no definite demarcation of two types of pupae into summer and winter varieties as claimed for *Stenopteryx* spp. from swallows.

In emerging, the imago pushes open the anterior end of the puparium which splits along a definite line around the case about one-fifth of the length of the puparium. When the fly first emerges the wings are still folded within the wing sheath. It is very active on its legs and within half an hour the wings have spread so that the insect is able to fly.

Of 69 flies which emerged from pupae in the laboratory during the 1941 series of experiments, 39 were females and 30 were males. Of the 19 flies which emerged in the 1942 experiment, 8 were females and 11 were males.

SUMMARY

Stilbometopa impressa, a parasitic fly on *Lophortyx californica vallicola*, does not exist away from its host more than 5 days. The normal life expectancy of an individual fly was not determined. It runs the risk of being devoured by its host, particularly in confined or crowded quarters.

The larvae are creamy white, ovoid, 5.5×4.5 mm, with a posterior spiracular plate 1 mm in diameter. The flies leave their host to deposit their larvae. Deposition may occur at any time during the day.

The puparium passes through a series of color phases until it is a shining black. This process may take as long as 16 hours. Imagoes emerge in from 61 to 162 days (in preliminary experiments as short a time as 29–43 days was recorded). The interval required for the pupal stage increases as winter advances but not in any definite progression.

Injury or contact with alcohol in the larval stage arrests development. Moistening with water does not prevent development. In a series of imagoes the sexes are produced in a ratio of approximately 1:1.

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NOTES ON THE LUNGWORMS OF NORTH AMERICAN LEPORIDAE

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In a recent paper (Goble and Dougherty, 1943) the incidence and identity of the lungworms in Varying hares in eastern North America was discussed. The nematode described as *Synthetocaulus leporis* by Boughton (1932) was re-described and renamed *Protostrongylus boughtoni*. Four subspecies of *Lepus americanus* Erxleben were listed as hosts of this parasite in eastern United States and Canada. Incidences observed from 1939 through 1942 ranged from 11 per cent in animals from New Brunswick to 48 per cent in those from Dickinson township in the Adirondack region. It is the purpose of this article to report further observations on *P. boughtoni*, made in New York during 1943, and to comment on Scott's (1943) recent article on lungworms in North American leporids.

OBSERVATIONS

Examination of the lungs of 31 hares (*Lepus americanus virginianus*) from Valcour Island (in Lake Champlain, near Plattsburg, N. Y.) revealed the presence of *Protostrongylus boughtoni* in 91 per cent of the adults (20 of 22) and 77 per cent of the juveniles (7 of 9). The infected juveniles were 5 weeks of age and older; the negatives were about 4 weeks old. The incidence of infection on Valcour Island is much greater than in other localities. This is probably due to the unusual environmental conditions prevailing there. Extensive predator destruction has resulted in an abnormally high game population in a habitat replete with the gastropod intermediate hosts of the parasite.

The same species of lungworm was also found in 6 out of 7 adult cottontails (*Sylvilagus floridanus mallurus*) and in 1 out of 3 juveniles. This is an unusual host record for this nematode, since it was not encountered in examinations of over 800 cottontails from other parts of New York state. The occurrence of more severe pathological conditions in the rabbits than those observed in hares also supports the view that the former is an abnormal host. Infection of cottontails in this locality is undoubtedly the result of the overlapping of their range with that of the Varying hares; intimate intermingling of these species is extremely rare in other localities.

DISCUSSION

To our knowledge the only other records of lungworms in the cottontail group are those of Scott and Honess (1932) and Scott (1943). In the latter paper Scott described *Protostrongylus sylvilagi* (emend. nov. from *P. sylvilagii*, Scott, 1943) and reported its occurrence in the Black Hills cottontail (*Sylvilagus nuttalli grangeri*) and White-tailed jack rabbit (*Lepus townsendi campanius*) of Wyoming. Since this article was unavailable to us at the time *P. boughtoni* was redescribed and since Scott was apparently unaware of Boughton's *Synthetocaulus leporis*, it seems desirable to point out the differences between *P. sylvilagi* and *P. boughtoni*, which are the only lungworms so far reported for leporids in North America.

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The males of *P. sylvilagi* differ from those of *P. boughtoni* chiefly in the length of the spicules (156–180 μ in the former, 260–320 μ in the latter). There are probably other distinguishing characters, particularly in the gubernacular complex, which are not evident in Scott's photomicrographs. The females of *P. sylvilagi* differ from those of *P. boughtoni* in the lack of a prominent provagina (present in the latter) and in the length of the vagina (597–1116 μ in the former, 1900–2400 μ in the latter).

Scott distinguished *P. sylvilagi* from *P. pulmonalis* (syn. *P. commutatus*) on the basis of the length of the crura (51–84 μ in the former, 33 μ in the latter). Studies on the latter species within the last 20 years have shown the crural length to be 40–58 μ which is within the size range given for the parts in *P. sylvilagi*. Since these two species seem to resemble each other in most particulars, restudy of the New World form, to determine its status, is indicated.

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PHYSIOLOGICAL OBSERVATIONS UPON A LARVAL EUSTRONGYLIDES. VII. STUDIES UPON SURVIVAL AND METABOLISM IN STERILE SURROUNDINGS

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In a previous paper of this series (von Brand and Simpson, 1942) it was shown that larval *Eustrongylides* occurring normally in cysts of *Fundulus* can be kept alive a long time in vitro in various media, provided that conditions remain sterile throughout the whole course of the experiments. A continuation and amplification of these studies seemed indicated, since, with the exception of Glaser and Stoll's (1938) and Stoll's (1940) work on *Haemonchus* larvae, no data are known on the behavior of parasitic nematodes kept in vitro under sterile conditions. It becomes more evident, however, from year to year that the development of sterile media for the maintenance of parasitic worms will represent a starting point from which the physiology of these organisms can be studied with a much greater degree of reliability than was hitherto possible.

In the present paper the following points have been investigated: influence of pH, various carbohydrates and lowering of the temperature from 37° to 20° C on the survival. Also studied were the rates of consumption of oxygen, sugar, and glycogen under various conditions.

MATERIAL AND METHODS

While in previous papers of this series the worm used was only tentatively designated as the larva of *Eustrongylides ignotus*, it can now be stated definitely that this identification was correct. The evidence will be presented in a later section.

As in the previous work the worms were isolated from cysts of *Fundulus heteroclitus*. The method of sterile extraction has been adequately described previously (von Brand and Simpson, 1942).

The media used in various series and their preparation will be discussed in the following sections. Only one point common to all series may be mentioned here, namely, that every isolated worm was kept in a separate tube.

RESULTS

1. *Influence of pH upon the survival.*—The worms were isolated in test tubes of standard size each containing about 10 cc medium (Bacto-Peptone 0.5 per cent, NaCl 0.5 per cent and glucose 0.5 per cent). The tubes were closed with cotton plugs and fitted with parafilm which effectively prevents excessive evaporation. The medium had been sterilized by filtration (through Berkefeld "N" filters) rather than by autoclaving in order to avoid destruction of the sugar at the higher pH values. The medium was replaced every four weeks with the usual precautions to avoid contamination. A number of control tubes containing no worms was always carried along. These showed in general only very small changes from the original pH.

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Column 3 of Table 1 represents the difference in pH between the tubes containing worms and the control tubes incubated at 37° C for identical periods. All pH measurements were carried out electrometrically.

A survey of the figures assembled in Table 1 makes it obvious that the optimal pH for survival in vitro lies around the neutral point. This is somewhat surprising since after extraction from the cyst and transfer to high temperature the worms are in a stage comparable to that of juvenile specimens beginning to establish themselves in the definitive host. As such, various species of herons may serve, and in these birds the worms become established in the glands of the fore stomach. The pH of the wall of the fore stomach, as determined by Mennega (1938) is strongly acid (pH 3.10) in starving herons and somewhat less acid (pH 4.14 to 5.75) in birds having received food from 45 minutes to 3½ hours prior to the determinations.

The figures shown in Table 1 are also a good proof for the extreme resistance of these helminths against the hydrogen ion, a fact already noted in previous studies concerning the rate of oxygen consumption at various pH (von Brand, 1943). The

TABLE 1.—*Survival of larval Eustrongylides ignotus in sterile surroundings at various pH.*
Medium: Bacto-Peptone 0.5 per cent, NaCl 0.5 per cent, glucose 0.5 per cent.
Temperature 37° C

Number of worms	pH at beginning	pH change after 4 weeks	Survival in days		
			Average	Minimum	Maximum
19	1.90	+ 0.20	21	7	48
18	3.77	+ 0.20	48	14	103
42	4.78	+ 0.10	62	20	115
40	5.74	- 0.09	84	14	179
27	7.16	- 0.28	114	32	225
23	7.94	- 1.08	84	36	151
25	9.40	- 1.35	50	2	97
31	11.30		A few hours	..	...

survival even under fairly extreme conditions is still better than that usually found in other parasitic worms under the best possible conditions.

Of some interest are the changes in pH that occurred during the course of the experiments. In the extreme acid range a slight increase in pH was observed, while in the slightly acid or the alkaline tubes a lowering occurred. This drop was the more marked the more alkaline the initial reaction of the medium. The compensation point was not exactly determined but can be assumed to lie somewhere in the vicinity of pH 5. Similar observations are at hand also for other nematodes. Whitlock, Link and Leasure (1939) found that *Strongylus* shifts solutions that are strongly acid or alkaline in the direction of the pH occurring in their normal habitat (pH 6.35 to 7.91). Gettier (1942) observed a similar trend in tubes in which *Camallanus scabrae* were kept, the compensation point in this case being somewhere between pH 5 and 6. The experiments of these authors, however, were not conducted with sterile worms. It is therefore not clear whether these changes were due to the worms themselves or to the bacteria developing in the solutions used. The present observations are the first definite indication that such pH changes can be brought about by the worms themselves. The chemical mechanism underlying them will require further studies.

2. *Influence of various carbohydrates on the survival.*—In a previous paper (von Brand and Simpson, 1942) we have pointed out that the addition of 0.5 per cent glucose to various basic media considerably increased the survival in vitro under

sterile conditions. It was therefore deemed of interest to test whether other carbohydrates would have a similar influence.

The technique used was the same as that described in the previous section with the obvious difference that in most series instead of glucose a corresponding amount of one of the other carbohydrates was added to the medium and that a control series without any sugar was carried along.

The results of these series are summarized in Table 2. It appears that the only carbohydrates that increased the length of life of the worms in a significant manner were glucose, xylose and perhaps inulin. It is rather curious that mannose, fructose and maltose, all sugars that can be utilized by such a great number of various organisms, had no similar influence.

The pH changes in all series were quite similar. While, as pointed out above, the mechanism of the acidification is not yet known, it should be kept in mind that

TABLE 2.—*Influence of carbohydrates on the survival of larval Eustrongylides ignotus in sterile surroundings. The carbohydrate concentration in the basic medium (Bacto-Peptone 1.0 per cent, NaCl 0.5 per cent) was in each case 0.5 per cent.*
Temperature 37° C

Carbohydrate	Number of worms	Extremes of survival in days	Average survival (M) and standard error (SE) in days	M carboh. - M contr.	CR*	Initial pH	pH change after 4 weeks
None (control) ..	30	12-118	65 ± 3.88	...	..	7.40	- 0.27
Cellobiose	29	22-105	61 ± 3.58	- 4	0.8	7.31	- 0.31
Xylose	27	45-125	84 ± 4.23	+ 19	3.3	7.20	- 0.34
Glucose	27	33-196	93 ± 7.32	+ 28	3.4	7.16	- 0.28
Mannose	24	43- 97	73 ± 3.17	+ 8	1.6	7.30	- 0.27
Laevulose	20	6-142	77 ± 7.51	+ 12	1.4	7.10	- 0.40
Maltose	30	14-203	71 ± 7.13	+ 6	0.7	7.20	- 0.20
Inulin	25	10-176	89 ± 8.39	+ 23	2.6	7.36	- 0.10

* Critical ratio = $\frac{M \text{ carboh.} - M \text{ contr.}}{\sqrt{SE(M \text{ carboh.})^2 + SE(M \text{ contr.})^2}}$. If the figure in this column is greater than 2, the average survivals are significantly different.

these observations do not necessarily indicate that it is not connected with the carbohydrate metabolism. As will be shown in a following section, larvae having no access to sugar in the medium, which obviously would also be the case if a non-utilizable sugar only were present, metabolize rather considerable amounts of glycogen from their body reserves.

3. *Survival at 20° C.*—All the experiments described so far were conducted at body temperature. It appeared essential to study the survival also at a temperature that was comparable to that in which the normal intermediate host, *Fundulus*, lives. The series described in this section were therefore conducted in a constant temperature room at 20° C.

For various reasons the technique used was somewhat changed. It seemed inadvisable to close the tubes with cotton and parafilm since at this lower temperature molds have a tendency to grow through the cotton plug. The tubes were therefore closed with a sterile rubber stopper. Since the use of rubber stoppers (#5) in place of cotton plugs is more likely to permit contamination during removal and replacement of fluid it was decided to avoid the periodic opening of the tube that accompanies a renewal of medium. Instead, larger tubes (25 × 150 mm) were used and each worm was immersed in approximately 30 cc medium. This left a considerable air space above the fluid. While it must be assumed that the oxygen tension decreased

and the carbon dioxide tension increased during the long periods of survival, no signs of asphyxiation were observed.

Two series were carried out and their results are summarized in Table 3. The survival at 20° C is obviously excellent. During the first year of observation only 3 worms died, corresponding to a death rate of only 12 per cent. After 2 years most of the worms kept in Bacto-Broth had died but over 80 per cent of the larvae isolated into Bacto-Proteose Peptone survived.

These periods exceed by far all that have been described for any parasitic worm kept in vitro. The worms were perfectly quiescent and curled up at the bottom of the tubes just as they are in the cysts of *Fundulus*. After having lived for nearly two years in the constant temperature room in the dark they were exhibited for an evening to the Helminthological Society of Washington. During this time they were exposed to a somewhat higher room temperature and to strong light, which caused the worms to become quite active, just as active in fact as freshly isolated specimens. The series are not yet completed and will be observed further. Unfortunately no suitable definitive host is available; it would have been of great interest to test whether these larvae still retain their infectivity.

TABLE 3.—*Survival of larval Eustrongylides ignotus at 20° C in sterile media. Series A: Bacto-Broth 1.6 per cent, NaCl 0.85 per cent, glucose 0.5 per cent. Series B: Bacto-Proteose Peptone 1.0 per cent, NaCl 0.85 per cent, glucose 0.5 per cent*

Number of worms at beginning	Number of worms living after specified month				
	6	12	18	24	30
Series A: 14	14	13	13	3	2
Series B: 12	12	10	10	10	7

4. *Sugar, glycogen and oxygen consumption in vitro.*—In order to study quantitatively some phases of the metabolism of the larvae in vitro, several facts had to be considered that necessitated some changes in the experimental procedure. If sugar was desired in the medium its concentration was kept at about 200 mgm per cent rather than at 500 mgm per cent because the method used for its determination (Hagedorn and Jensen, 1923) is more easily carried out in the former range. The amount of medium introduced into each tube was exactly 10 cc instead of the approximate amounts used in the other series. The medium was distributed into the tubes immediately after Berkefeld filtration from a filter flask fitted with a sterilized dispensing apparatus, the essential feature of which was a 50-cc burette, graduated to 0.1 cc. The test tubes were closed with sterile rubber stoppers in order to avoid any evaporation. While cotton plugs covered with parafilm are quite sufficient to prevent any large degree of evaporation they do not guarantee an exact maintenance of the volume which was of paramount importance for the intended experiments. Half of the series were conducted at 37° C and half at 20° C.

After the intervals stated in Table 5 both the control tubes without worms and the experimental tubes were opened for the chemical determinations. Before the samples for the latter were taken out, however, samples were withdrawn in order to test whether the sterility of the medium had been maintained. These bacteriological controls were carried out as follows: By means of individual sterile pipettes fitted with rubber bulbs, a sample was removed from each tube and a portion inoculated

into a tube of glucose broth and the remainder into a tube of sodium thioglycollate medium. The amount of inoculum was approximately 0.5 cc. These tubes were incubated at 37° C for 48 hours or longer. All tubes proved to be sterile.

Immediately after the bacteriological sampling the sugar content of each tube was determined. It was found that the changes occurring in the control tubes during the periods of incubation were negligible and entirely within the normal range of error of the method. The greater changes observed in the tubes that contained worms could therefore safely be attributed to the metabolism of the latter.

After the sugar samples had been withdrawn the worms were removed from the tubes and a part of the remaining medium was used for a pH determination.

The worms were weighed individually and their oxygen consumption was determined in a Warburg apparatus, using the same technique as described in previous papers (von Brand, 1942, 1943). These determinations were not carried out with the single worm specimens, since the manometer readings would then have been too small to be accurate, but two or three worms were used together. The fluid introduced into the vessels had been taken from the experimental tubes in which the worms

TABLE 4.—*Glycogen content and oxygen consumption of freshly isolated larvae of Eustrongylides ignotus. Average figures*

Series	Number of worms	Average weight of single worm mgm	Medium*	Temp. (° C)	Oxygen consumption in cmm per 1 gm and 1 hour	Glycogen in per cent of fresh weight
Control 1 . . .	30	80	A	37	166	8.24
" 2 . . .	32	74	B	37	157	8.51
" 3 . . .	36	88	C	37	148	...
" 4 . . .	16	73	A	25	82	...
" 5 . . .	15	60	B	25	95	...
" 6 . . .	41	76	C	24	67	8.59

* A: Bacto-Broth 0.8 per cent, NaCl 0.6 per cent, glucose 0.2 per cent.

B: NaCl 0.8 per cent, glucose 0.2 per cent.

C: NaCl 0.85 per cent.

had lived previously. The oxygen determinations with those worms that had been kept at 37° C were carried out at that same temperature, those with the worms of the 20° C group were performed at 24 or 25° C, since the waterbath had to have a somewhat higher temperature than the surrounding air in order to function properly. In a previous paper (von Brand, 1943) it has been shown that the temperature coefficient in this range is 3.55 and this figure was used in calculating the actual oxygen consumption during the experimental period at 20° C. Most of the oxygen determinations were carried out for periods of 4 hours with half-hour readings in the case of the 37° series and hourly readings in that of the 20° group.

These determinations were followed by determinations of the glycogen content of the worms according to the micro-modification of Pflüger's method as described by von Brand (1936).

It was unfortunately not possible to determine either the weight or the rate of oxygen consumption of these worms at the start of the experiments since during such procedure bacterial contamination would have been unavoidable. Although some changes in weight must undoubtedly have occurred it does not appear likely that they were very pronounced. The average weight of the worms at the termination of the experiments was about the same as that of worms recently isolated from fish, as a comparison of the figures in Tables 4 and 5 shows. In order to get an initial value for the oxygen consumption and the glycogen content, series of freshly

TABLE 5.—*Glycogen content and oxygen consumption of larval Eustrongylides ignotus kept in vitro under sterile conditions for specified periods and sugar content of the medium. Average figures*

Series	Number of worms	Average weight of single worm	Temperature	Medium*	In vitro period in days	Sugar content at end of in vitro period		Oxygen consumption		Glycogen per cent of fresh weight
						mgm Control tubes	mgm Worm tubes	Temp.	mm O ₂ /1 hr.	
		<i>mgm</i>	<i>° C</i>					<i>° C</i>		
1	16	66	37	A	23	22.4	21.0	37	86	7.00
2	18	84	37	A	39	22.3	20.4	37	92	5.08
3	22	91	37	B	24	20.2	18.2	37	97	6.41
4	18	89	37	C	21	...	...	37	108	6.14
5	12	85	37	C	39	...	...	37	79	4.75
6	10	65	20	A	130	21.1	20.4	24	56	9.22
7	12	101	20	A	264	21.4	20.4	25	59	6.23
8	10	93	20	B	108	20.4	17.8	25	51	8.29
9	16	93	20	B	250	20.8	16.7	25	58	6.34
10	16	82	20	C	146	...	...	24	38	5.53
11	16	71	20	C	256	...	...	25	47	2.86

* A: Bacto-Broth 0.8 per cent, NaCl 0.6 per cent, glucose 0.2 per cent.

B: NaCl 0.8 per cent, glucose 0.2 per cent.

C: NaCl 0.85 per cent.

isolated worms were used in which the oxygen consumption was determined at the same temperatures and in the same media that were used for the experimental lots. The glycogen figures of the initial series were very similar and their mean value, 8.45 per cent, is thought therefore to be representative of the initial glycogen content of the experimental worms.

The results of the experimental series are summarized in Tables 5 and 6, the former table being essentially a summary of the analytical data, the latter showing the results of the experiments calculated per 1 gm and 1 day.

If a comparison is made of the rates of oxygen consumption of the freshly isolated and the experimental worms (Tables 4 and 5) it becomes obvious that they are smaller in the latter. In a previous paper (von Brand, 1942) it was shown that the oxygen consumption declines rather rapidly after removal of the worms from the fish. A comparison of the rates of the series kept at the same temperature and in the same medium for various periods, at 20° for more than 8 months, shows that they remained quite constant. Only the latter figures were therefore used for the calculation of the average oxygen consumption (Table 6). It is believed that this neglect of the initial value introduced no great error.

TABLE 6.—*Data of Table 5 calculated per 1 gm worm and 1 day. Mean values for sugar, glycogen and oxygen consumption*

Series	Sugar consumed		Total carbohydrate consumed per day	Oxygen required for total oxidation of carbohydrates	Oxygen consumed per day	Oxygen required for total oxidation in per cent of consumed O ₂
	From medium	Derived from glycogen				
	<i>mgm</i>	<i>mgm</i>	<i>mgm</i>	<i>mgm</i>	<i>mgm</i>	
1	0.92	0.70	1.62	1.72	2.94	58
2	0.55	0.96	1.51	1.61	3.16	51
3	0.92	0.94	1.86	1.98	3.33	59
4	...	1.22	1.22	1.30	3.70	28
5	...	1.05	1.05	1.12	2.72	24
6	0.08	+ 0.06	0.02	0.02	1.15	2
7	0.04	0.09	0.13	0.14	1.22	11
8	0.26	0.02	0.28	0.30	1.05	29
9	0.18	0.09	0.27	0.29	1.33	22
10	...	0.22	0.22	0.23	0.78	29
11	...	0.24	0.24	0.26	0.86	30

It is quite apparent that the worms are capable of utilizing glucose that is present in the medium. A study of the analytical data from the series kept in the same medium for various lengths of time indicates however that this consumption takes place primarily during the first weeks. Later on it seems to have ceased entirely in experiments 2 and 7, but it was still quite pronounced in series 9. As a consequence the overall daily sugar consumption as computed in table 6 appears usually smaller in the longer lasting experiments.

In the 37° series (series 1 to 3) no difference in sugar consumption from the medium occurs whether the medium contained peptone or not, in the 20° series, however, it was considerably greater in the latter case.

In all series, with the exception of No. 6, some glycogen disappeared from the body. It is characteristic that in the series kept in the same medium for various periods at the same temperature relatively more glycogen disappeared during the longer incubation periods than during the shorter ones. Consequently the overall daily glycogen consumption is greater during the former—exactly the opposite of the case where sugar is present in the medium. Both phenomena are of course connected. At times in which the worms consume carbohydrate from the medium less glycogen has to be mobilized from the body reserves in order to cover the carbohydrate needs of the organisms. In cases where no sugar was present at all in the medium the glycogen consumption was the greatest (series 3 and 4, 10 and 11) and, what is especially characteristic, the daily consumption was about constant regardless of the duration of the experimental periods, depending in its intensity only on the temperature.

An actual increase in glycogen occurred only in series 6. It was relatively small and may not be real. The worms of this lot may have had initially a glycogen content surpassing the average.

The total carbohydrate consumption (sugar derived from glycogen and, where present, from the medium) is obviously a composite between the different behavior of the two fractions. It is noteworthy that the differences between the total daily carbohydrate consumptions that exist between identical series carried on for different periods are small and obviously within reasonable limits of error. This indicates that the carbohydrate consumption remained stable at 37° for a minimum of 5 to 6 weeks and at 20° for at least 8 months. This should mean that the worms remained quite normal outside their hosts at least for these periods.

An attempt has been made (Table 6) to determine whether the carbohydrate metabolism is an important part, quantitatively, of the total metabolism by calculating how much of the daily oxygen consumption is used for sugar oxidation. Several difficulties arise that make such a calculation somewhat insecure and the figures assembled in the last column of table 6 may therefore be open to future readjustment. In the first instance as has already been explained, the oxygen consumption figures are derived only from a final determination at the end of the experiments and one may doubt whether they can be used for an average of the whole period. In the second place it has been found previously (von Brand, 1942), that, at least in freshly isolated worms, the respiratory quotient is slightly above 1. This indicates probably that some incomplete oxidations are going on even at the oxygen tension of the atmospheric air. This may even be more pronounced than appears on the face of it. If, as the present figures indicate, the metabolism of the larvae in question is not a pure

carbohydrate metabolism the purely oxidative respiratory quotient would obviously lie below 1 and the increase of the overall quotient above 1 would indicate a greater participation of partial oxidations or fermentations. Provided that the mother substance which they attack is carbohydrate, as happens in many other cases, the oxygen actually used in the carbohydrate breakdown would be less than if one assumes total oxidation of the sugar.

Keeping these limitations in mind we may try to evaluate the data. It would appear then that in media containing sugar and kept at 37° about half of the total oxygen is used in the carbohydrate metabolism (series 1, 2 and 3), while in those lacking sugar (series 4 and 5) the amount is only about 25 per cent. The situation is different in the 20° series. In no case was more than 30 per cent of the consumed oxygen attributable to the carbohydrate metabolism. Only an exceedingly small amount of it could be referred to sugar oxidation if the medium contained peptone (series 6 and 7), perhaps an indication that this latter substance can also be utilized.

It is believed that the figures, despite their admitted insecurity, are an indication that the metabolic processes are different at low and high temperatures. This result is perhaps not too surprising. Previous work (von Brand, 1942) has indicated that the worm must be able to cover all its oxygen needs in the fish; once it is in the final host it may have more difficulty in doing so. It is, however, a general feature that with restricted oxygen supply the carbohydrate metabolism comes more and more into the foreground. It would appear possible that the mechanism that brings about the transition from one metabolic type to the other is the temperature, since the temperature of both hosts is characteristically different. Another indication that the metabolic processes may be different at different temperatures has already been found previously (von Brand, 1943). It was shown that the temperature coefficient of the oxygen consumption is 3.55 at low temperatures (up to 27° C) while above that limit it is only 1.48.

5. *Identification of the larvae.*—*Eustrongylides* larvae of different species are very similar and a specific identification cannot be given with absolute certainty. Dr. B. C. Chitwood, to whom the worms in question were submitted several years ago, identified them therefore only tentatively as *Eustrongylides ignotus*.

It now happened that one of the larvae, belonging to series 2 described in the last section, molted in vitro into an adult male. The specimen was preserved in formalin and sent to the Bureau of Animal Industry where it was studied by Mr. J. T. Lucker. He reports that, although minor differences in the arrangement of the papillae are present compared with the original description, it is *Eustrongylides ignotus*. The papillae correspond exactly to those of specimens collected by Cram (1933) from *Nycticorax nycticorax hoactli* which had been identified by her as *Eustrongylides ignotus*.

The worm was then sectioned in order to find out whether the reproductive organs had matured. However no definite signs of sperm formation was found; it was apparently still a juvenile specimen.

No definite reasons can be given why this particular animal molted while the far more than 1,000 specimens that have been kept isolated during the present and previous studies did not do so. It may be pointed out that, contrary to most other experiments, the tubes of this series were closed by means of rubber-stoppers and had a sugar concentration of only 0.2 per cent. The temperature, as in most other series,

was 37° C. Whether a lowered oxygen tension or an increased carbon dioxide tension may have played a role will have to be decided by further studies. It is of interest in this connection, that Stoll (1940) found that the first parasitic ecdysis of *Haemonchus contortus* is definitely favored by a lowered oxygen tension.

SUMMARY

1. All experiments were performed under strictly sterile conditions.
2. The larvae of *Eustrongylides ignotus* survive longest when the medium has a pH around the neutral point, but they survive relatively well also in quite acid and alkaline surroundings.
3. In very acid surroundings the worms shift the pH of the medium slightly towards the alkaline side. In but slightly acid or alkaline media they are responsible for an acidification.
4. The survival in vitro is favored if the medium contains either xylose, glucose or perhaps inulin. No significant influence was found with cellobiose, fructose, mannose or maltose.
5. The survival at 20° C is much longer than at 37°. The worms lived in the former temperature over 2½ years in vitro.
6. Quantitative data are presented concerning the sugar, glycogen and oxygen consumption for worms kept in vitro at 37° for periods up to 5 weeks and at 20° for periods up to 8 months. It is pointed out that at the former temperature relatively more oxygen seems to be used in the carbohydrate metabolism than in the latter.
7. Due to the fact that one specimen molted in vitro into an adult male a specific identification of the worms became possible. The previous tentative identification as the larvae of *Eustrongylides ignotus* could be confirmed.

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AMERICAN SOCIETY OF PARASITOLOGISTS

PRELIMINARY ANNOUNCEMENT OF THE NINETEENTH ANNUAL MEETING

Monday, Tuesday, and Wednesday, September 11-13, 1944, Cleveland, Ohio

In accordance with the action of Council, the American Society of Parasitologists will convene for a three-day program in conjunction with the meeting of the American Association for the Advancement of Science in Cleveland, Ohio, from September 11 to 13, 1944.

A call for papers has already been sent to all members of the Society. In order that the abstracts be published and mailed to the membership before the meetings, it is necessary that they be received in the office of the Secretary not later than June 15, 1944. Members are hereby reminded of this date, and are requested to submit papers promptly.

So far as can be anticipated, the program will be arranged as in previous years, although some changes may be necessary because of circumstances resulting from the War. In the event that war conditions in September make a general meeting impractical, it is possible that regional meetings may be substituted or that the proposed meeting will have to be postponed or cancelled.

JAMES T. CULBERTSON,
Secretary.